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BACTERIAL AEROSOLS AND METHODS OF STUDYING THEM IN SANITATION MICROBIOLOGY

V. S. Kiktenko, et al

Foreign Technology Division
Wright-Patterson Air Force Base, Ohio

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By: V. S. Kiktenko, S. I. Kudryavtsev, N. I. Chugunov, M. I. Pushchin

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V. S. Kiktenko, S. I. Kudryavtsev,
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U. S. BOARD ON GEOGRAPHIC NAMES TRANSLITERATION SYSTEM

Block	Italic	Transliteration	Block	Italic	Transliteration
А а	*А а*	A, a	Р р	*Р р*	R, r
Б б	*Б б*	B, b	С с	*С с*	S, s
В в	*В в*	V, v	Т т	*Т т*	T, t
Г г	*Г г*	G, g	У у	*У у*	U, u
Д д	*Д д*	D, d	Ф ф	*Ф ф*	F, f
Е е	*Е е*	Ye, ye; Ë, ë*	Х х	*Х х*	Kh, kh
Ж ж	*Ж ж*	Zh, zh	Ц ц	*Ц ц*	Ts, ts
З з	*З з*	Z, z	Ч ч	*Ч ч*	Ch, ch
И и	*И и*	I, i	Ш ш	*Ш ш*	Sh, sh
Й й	*Й й*	Y, y	Щ щ	*Щ щ*	Shch, shch
К к	*К к*	K, k	Ъ ъ	*Ъ ъ*	"
Л л	*Л л*	L, l	Ы ы	*Ы ы*	Y, y
М м	*М м*	M, m	Ь ь	*Ь ь*	'
Н н	*Н н*	N, n	Э э	*Э э*	E, e
О о	*О о*	O, o	Ю ю	*Ю ю*	Yu, yu
П п	*П п*	P, p	Я я	*Я я*	Ya, ya

* ye initially, after vowels, and after ъ, ь; e elsewhere. When written as ё in Russian, transliterate as yë or ë. The use of diacritical marks is preferred, but such marks may be omitted when expediency dictates.

The study of bacterial contamination of the air in industrial areas, hospitals, and dwellings is taking on ever greater significance.

The significance of bacterial seeding of the air in such industries as the production of antibiotics and other biologicals, the production of serum and vaccines, the food industry, etc., frequently has a decisive influence on the operation of these establishments. No less important is the determination of bacterial contamination in living areas, children's institutions, and hospitals with respect to sanitation and hygiene characteristics of the operation of such institutions.

Study of bacterial contamination of air is particularly important from the point of view of rational development of measures to combat and prevent infections spread by droplets in the air. This work presents information on the present state-of-the-art in sanitation bacteriological study of the air.

The materials presented cast fairly detailed light on both the basic laws characterizing bacterial aerosols and on the methods of studying the air. Practical recommendations are presented with consideration of application of these methods and equipment in practice by sanitation bacteriological laboratories.

The book is intended for scientific workers and other medical specialists in preventive medicine, veterinary science, and biology working in the field of combatting and preventing airborne infections (sanitation bacteriology, epidemiology, specialists in infectious diseases).

The book is illustrated by 49 figures and 14 tables.

FOREWORD

At the present time the problem of biological and, primarily, sanitation-bacteriological contamination of the air in the open atmosphere and also in buildings with various functions is the object of broad study both in various fields of biology (aerobiology) and medicine and also in many branches of industry. Knowledge about the nature of the formation and behavior of bacterial aerosols is necessary first of all for workers in preventive medicine studying questions of the propagation of causative agents of certain infectious diseases through the air and also studying routes of transmission of intrahospital infections. The systematic study of bacterial contamination of the air in industry is of major significance, since the conditions of production technology require a definite degree of sterility of the air (production of biologicals, the food, dairy, and meat industries, and other branches of industry).

Naturally, quantitative and qualitative characteristics of "biological contamination" of the air both indoors and out can be adequately studied only through the use of effective methods of detecting aerosol particles containing bacterial and viral cells. Development of such methods and instruments and also their effective practical application can be realized only with adequate knowledge in the field of physical and biological laws governing

the behavior of air-dispersed systems in general and of bacterial aerosols in particular. Therefore, in this book the authors have considered it necessary first of all to outline briefly the available information on the laws which characterize the physicochemical properties of aerosols.

In connection with the fact that measures for quantitative evaluation of bacterial contamination of the air in the sanitation field are based mainly on the physical principles for determining particulate concentration of aerosols of an inorganic nature, the book gives a brief description of these research methods.

The book presents in greatest detail data from Soviet and foreign science on contemporary methods of studying the sanitation bacterial contamination of the air (biological aerosols): a systematized survey of apparatus is presented in accordance with a proposed classification of instruments and bacterial traps; practical recommendations are presented on the possibility and advisability of using one or another method to study bacterial contamination of the air, depending upon the stated goals and the problems.

The authors do not pretend to a complete description of all existing varieties of bacterial traps and instrumentation, and therefore major attention is paid in the book to outlining the principles underlying the operation of the various instruments.

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CHAPTER I

BACTERIAL AEROSOLS AND THEIR SANITATION AND EPIDEMIOLOGICAL SIGNIFICANCE

1. General Concepts on Aerosols

Intensive investigation of aerosols (air dispersed systems) presently occurring is explained by the enormous importance of knowledge about air-dispersed systems for research in many processes occurring in nature, science, engineering, agriculture, and medicine, and with particular reference to the study of bacterial aerosols.

The study of bacterial aerosols is of great theoretical and practical significance in connection with the broad distribution of airborne droplet infections, possible bacterial contamination of the air in various establishments and in stock-breeding farms, the use of aerosols in therapeutic and prophylactic medicine and also in sanitation and hygiene practice during development of qualitative and quantitative methods of evaluating bacterial seeding of the air.

The term aerosols is applied to dispersed systems with a gaseous medium and a solid or liquid dispersed phase. Under natural and production conditions aerosols are obtained during processes of dispersion and condensation (sublimation and coagulation).

The dispersion method is used to obtain aerosols with fine crushing and atomization of solid or liquid bodies, their conversion into the suspended state by air flows and by vibration, and also during mixing and pouring of fluids, with passage of a gas through a liquid, etc. Thus, extremely fine suspensions of aerosols (fogs) are formed at points of impact of waterfalls or during powerful ocean surf action.

At present no single widely accepted classification of aerosols exists. As is known, aerosol particle sizes fall in very wide limits - from a few millimeters (snowflakes, raindrops) down to 10^{-7} cm (0.001 μm). Such a broad range of variation in aerosol particle size has led to the need to break aerosols down with respect to degree of dispersion into coarsely dispersed (with radius greater than 10^{-4} cm) and highly dispersed, with radii below 10^{-4} cm (N. A. Fuks, 1955).

Depending on the degree of uniformity with respect to degree of dispersion, aerosols are broken down into monodispersed and polydispersed systems.

One of the most widely used classifications of aerosols with respect to dimensions is that proposed by V. Gibbs* (1930), according to which the following classes are distinguished.

1. Dust. Particle diameter exceeds 10^{-3} cm, i.e., 10 μm. Such particles settle out of unmoving air with increasing speed; they are not diffused.

2. Clouds or fogs. Particle diameter varies from 10^{-3} to 10^{-5} cm, i.e., from 10 to 0.1 μm. Such particles settle out of

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*Transliterated in Russian; exact spelling not found - Translator.

unmoving air at a constant rate, depending on size and specific gravity (according to Stokes law); the particles diffuse weakly.

3. Smokes. Particles are 10^{-5} to 10^{-7} cm in size, i.e., 0.1 to 0.001 μm. These particles, which approach molecules in size, are in intensive Brownian motion. They diffuse quite energetically and they either virtually do not settle out of unmoving air or settle out very slowly.

While retaining the name "dust" for the first form of the given classification of aerosols, for a coarse suspension, Yu. I. Veytser and G. P. Luchinskiy (1947) combine the last two forms of aerosols, representing true colloidal systems, under the general name "smokes."

They propose that a subsequent division within the area of true colloidal-chemical systems be based on the aggregate state, and they apply the name "smokes" to aerosols with a solid dispersed phase, while aerosols with liquid particles are called fogs.

A. G. Amelin (1961) also subdivides aerosols in terms of the form of dispersed phase and indicates that aerosols whose dispersed phase consists of liquid droplets are called fogs, while in the case of a solid dispersed phase the term smokes is applied. Separating aerosols with respect to degree of dispersion, this author indicates that "dispersion of the majority of aerosols is substantially lower than that in colloidal systems and is close to the dispersion of the emulsions and suspensions; therefore it is more correct to call aerosols aerosuspensions (smokes and dust) and aeroemulsions (fog)." In fogs the particles take the form of spherical droplets, while in smokes and dusts they are crystalline particles or aggregates of various shapes.

Noting the rather vague nature of the ordinary classifications of aerodispersed systems, R. Whitlow-Grey and H. Patterson (1934) include in the category of smokes all aerosols which consist of particles which settle out slowly under the action of gravity.

From the epidemiological and epizootological point of view major importance attaches to the classification of aerosol particles in size, in connection with their differing ability to penetrate more or less deeply into the respiratory tract and to be retained there. In this connection dust is broken down into particles with sizes greater than 50 μm, particles of 50 down to 10 μm, and particles smaller than 10 μm.

This classification is based on the fact that particles larger than 50 μm in size are retained in the upper portions of the respiratory tract (nose, nasopharynx, trachea, large bronchi). Although they penetrate deeper into the respiratory tract, particles of 10 to 50 μm size reach the lung tissue in very small quantities; finally, fine dusts less than 10 μm in size can penetrate into the deepest branches of the lungs - the bronchioles and alveoli - and represent the greatest danger to the organism (Table 1) (A. I. Burshteyn, 1934).

Classifications of aerosols proposed by different authors are compared in Table 2.

Besides the common but not yet established classifications of aerosols there are a number of special classifications - for example, classification by the ability of aerosols to penetrate the respiratory tract and lungs, the Atterberg scale, etc.

Table 1. Classification of aerosol particles with respect to size as a function of ability to penetrate and be retained in the respiratory tract (after A. I. Burshteyn, 1934).

Particle size (μm)	Ability of particles to penetrate and be retained in areas of the respiratory tract
Less than 0.2	Reach the lungs and are retained in them
From 0.2 to 5	Easily carried to the lungs
Over 5 to 10	Can enter lungs but are rarely found in them
Over 10 to 50	Usually do not reach the lungs; retained in the upper respiratory areas and in bronchi, are gradually carried out
Over 50	Do not reach the lungs; retained in upper respiratory areas, easily expelled

The liquid or solid substance which comprises the dispersed phase of the aerosol possesses a number of properties which are not inherent to it in the nondispersed state or which occur then to a much less sharply expressed degree. This is true of both physical and physicochemical properties of the substances.

The peculiarities of substances making up the dispersed phase of aerosols are brought about mainly by the great differences in densities of the dispersed phase and the dispersion medium and also by the low viscosity of the latter. These factors exert an essential influence on the basic characteristics of an aerosol (degree of dispersion and concentration).

All aerosol systems are more or less unstable. The degree of stability is dependent mainly on particular features inherent to the aerosol systems themselves. Under natural conditions the stability of systems is influenced largely by a number of factors external to the system - for example, the state of the atmosphere and the nature and relief of the terrain.

Table 2. Classification of aerosols proposed by various authors.

Classification of aerosols	Type of aerosol	Other names	Tentative limits of size (diameter) of particles (cm)
By composition of dispersed phase	a) With solid dispersed phase: dust		$1 \cdot 10^{-4}$–$1 \cdot 10^{-2}$
	smoke		$1 \cdot 10^{-7}$–$1 \cdot 10^{-4}$
	b) With liquid dispersed phase: fogs		$1 \cdot 10^{-3}$–$1 \cdot 10^{-2}$
	c) With solid and liquid dispersed phase (smokes – products of incomplete combustion of fuels, hygroscopic substances, etc.)		
By origin	a) Condensation (fogs, smokes)	Dispersion-condensation (N. A. Fuks, 1955)	$1 \cdot 10^{-7}$–$1 \cdot 10^{-2}$
	b) Dispersion (dusts, fogs)	Primary (formed during condensation and sublimation)	$1 \cdot 10^{-4}$–$1 \cdot 10^{-1}$

Table 1 (continued)

		Coagulation	$1 \cdot 10^{-5}$–$2 \cdot 10^{-5}$
		Dispersion (V. Fett, 1961)	
By degree of dispersion	a) Coarsely dispersed		$1 \cdot 10^{-4}$ and above
	b) Highly dispersed		$1 \cdot 10^{-4}$–$1 \cdot 10^{-7}$ and below
By degree of uniformity of particle size	a) Monodispersed	Uniform or homogeneous	
	b) Polydispersed	Nonuniform or heterogeneous (V. Fett, 1961)	
	c) Isodispersed		

The stability of aerosol systems also depends on conditions which characterize the physical state of the dispersed system: degree of dispersion, particle and weight concentration of the dispersed phase, the shape, density, and structure of the dispersed particles, temperature, pressure, and the electrical state of the system.

The basic processes which determine the state of dispersed systems are sedimentation, diffusion, coagulation, and condensation.

Sedimentation is the process by which particles settle out under the influence of gravity. The rate of settling of aerosol particles in an unmoving medium is determined basically by particle dimensions. In addition, the rate of sedimentation depends on the density of the dispersed phase, the density and viscosity of the medium, the shape of the particles (spherical and nonspherical), the magnitude of the electrical charge of the particles, and other factors. The fundamental equation for determining sedimentation rate is the Stokes formula (1911) (cited by K. Spurny et al., 1964), derived for conditions of settling of solid spherical bodies in a liquid under the assumption that with a particle size less than 100 μm the air which is the dispersion medium can be regarded as a viscous fluid.

The Stokes formula has the form

$$V_s = \frac{2r^2 \cdot g(\rho - \rho_1)}{9 \cdot \eta}, \qquad (1.1)$$

where g is the acceleration of gravity in centimeters per second; ρ is the density of particles in grams per cubic centimeter; ρ_1 is the density of the medium in grams per cubic centimeter; r is the radius of the particle in centimeters; η is the viscosity of the medium in grams per cm per second; and V_s is the rate of uniform settling in centimeters per second.

The obtained equation is valid under the following assumptions: the shape of the falling particle is spherical; the medium in which the fall occurs is uniform or, more accurately, includes inhomogeneities which are small as compared with the dimensions of the spherical particle; there is no sliding of particles over the surface of the medium and the rate of travel of a spherical particle is so small at the total resistance to movement occurs not because of inertia of the medium but only as a result of friction; the movement of a particle is steady - i.e., the rate of fall is constant; the settling particle is solid.

The Stokes formula makes it possible to calculate the rate of deposition of particles whose degree of dispersion lies in a narrow interval. However, by the introduction of appropriate corrections or by yielding somewhat in terms of accuracy this formula can be applied to calculate the rate of deposition for particles with a radius varying from 0.1 up to 50 μm (N. A. Fuks, 1955). V. Fett (1961) indicates that the Stokes formula can be used to calculate the rate of deposition for particles with a radius of $1 \cdot 10^{-2}$ to $2.5 \cdot 10^{-4}$. The values of the corrections to the Stokes formula are, as a rule, not great. In particular, the correction during calculation of the rate of deposition of particles which are liquid rather than solid (water fogs) in air amounts to 0.7%, while that for oil fogs is merely hundredths or thousandths of a percent. In view of the fact that the density of air ($\rho_1 = 1.293 \cdot 10^{-3}$ g/cm^3) is a small quantity as compared to particle density ($\rho = 1$ g/cm^3), it can be ignored.

Then the Stokes formula will take the form:

$$v_0 = \frac{2r^2 \cdot g \cdot \rho}{9\eta}. \qquad (1.2)$$

Here is an example of calculating deposition rate of particles by the Stokes formula: let it be required to calculate the rate of settling in air of particles 50 μm in diameter ($r = 2.5 \cdot 10^{-3}$ cm).

From the conditions of the problem we have the following: density of particles $\rho = 1$ g/cm^3, viscosity of air $\eta = 18.3 \cdot 10^{-5}$ g/cm·s; acceleration of gravity $g = 980$ cm/s^2.

Substituting these values into the Stokes formula, we obtain

$$V_s = \frac{2}{9} \cdot (2{,}5 \cdot 10^{-3})^2 \cdot \frac{1}{18{,}3 \cdot 10^{-5}} \cdot 980 = 7{,}5 \text{ cm/s}.$$

Consequently, the rate of settling for particles 50 μm in diameter comprises 7.5 cm/s.

Cunningham (1910) introduced a correction into the Stokes formula which considers molecular discontinuity of the air. In the literature this correction is frequently called the Cunningham correction, and the formula is called the Cunningham-Stokes formula. It has the following form

$$V_s = \frac{2r^2 \cdot g(\rho - \rho_1)}{9\eta}\left(1 + A\frac{\lambda}{r}\right). \qquad (1.3)$$

where A is the Cunningham constant, whose value equals 1.246; λ is the tentative mean free path of a molecule of air ($0.653 \cdot 10^{-5}$ cm).

The Cunningham formula is true only at small values of the quantity λ/r and, as a rule, is used to calculate the settling rate for particles whose radius is about 0.05-10 μm.

With an increase in the size of the settling particles the quantity $A(\lambda/r)$ goes to zero and in this case formula (1.3) is converted into the Stokes formula (1.1).

Values of settling rate for particles as calculated by the Stokes formula lie below the values of the rates calculated by

the Cunningham-Stokes formula, with the difference being 146% for particles 0.1 μm in radius, 15% for 1 μm radius particles, and 1.5% for particles with a radius of 10 μm.

To give a clear picture of the application of the Cunningham-Stokes formula we will present an example of calculating the rate of settling for particles with a radius of $1 \cdot 10^{-4}$ cm:

$$V_s = \frac{2}{9} \cdot (1 \cdot 10^{-4})^2 \cdot \frac{1 - 1{,}29 \cdot 10^{-8}}{18{,}3 \cdot 10^{-5}} \cdot 980 \left[(1 + 1{,}246) \cdot \frac{0{,}633 \cdot 10^{-5}}{1 \cdot 10^{-4}} \right] = 0{,}013 \text{ cm/s}.$$

Consequently, the settling rate for particles 2 μm in diameter comprises 0.013 cm/s.

The Cunningham-Stokes formula found broad application for determining dimensions of particles by a method based on determining the sedimentation rate of the particles in an ultramicroscopic cell.

N. A. Fuks and A. G. Sutugin (1961) applied this formula for particles with dimensions of about 1 μm.

The rate of settling for particles of nonspherical form is also expressed through the Stokes formula, with numerical coefficients dependent upon the particle shape being introduced into the expression. Experimental and theoretical works in determining the settling rate for nonspherical particles were examined in detail and generalized by N. A. Fuks (1955, 1961).

Particles less than 0.1 μm in size, subjected to nonuniform impacts of gas molecules, accomplish disordered Brownian motion; in this case they settle out very slowly (particle radius 0.1-0.05 μm) or do not settle at all, but instead take part in the molecular motion of the gas (particle radius 0.001 μm and less) and diffuse in all directions.

Thus, diffusion should be regarded as a phenomenon caused by Brownian motion and reduced to the fact that highly dispersed particles of an aerosol strive to achieve uniform distribution in space. Gravitational forces prevent such a distribution.

The degree of dispersion of aerosol particles is a labile quantity. Under the influence of the most widely varied causative factors, with the passage of time an aerosol becomes polydispersed; this leads to more rapid sedimentation of enlarging particles and to destruction of the aerosol system. Especially rapid aggregation of particles is recorded in highly dispersed aerosols in the initial period of their appearance.

The process of adhesion or accumulation of aerosol particles during collisions with one another, called coagulation, occurs as a rule due to Brownian motion of particles or thanks to the superposition on the Brownian motion of ordered motion of particles toward one another under the action of various forces (gravitational, electrical, etc.).

A theory of the thermal coagulation of aerosols with spherical particles was developed by M. V. Smolukhovskiy (1936). During development of the theory of coagulation of an isodispersed aerosol the fact that coagulation of particles occurs at each collision between them was taken as the initial assumption.

N. A. Fuks indicates that this assumption "has a solid theoretical and experimental basis" (for particles 0.1-1 μm in size).

The fundamental equation of coagulation in differential form is

$$\frac{dn}{dt} = -K_1 n^2, \qquad (1.5)$$

An interval form,

$$n = \frac{n_0}{1 + K_0 n_0 t}, \qquad (1.5)$$

where n_0 is the initial concentration of particles; n is the particle concentration after time t; K_0 is the coagulation constant expressed by the formula: the quantity $K_0 = 8\pi rkTB$ or, proceeding on the basis of the Cunningham formula for mobility,

$$K_0 = \frac{4kT}{3\eta}\left(1 + A\frac{\lambda}{r}\right),$$

where k is the Boltzmann constant equaling $1.38 \cdot 10^{-6}$ erg·deg^{-1}; T is the absolute temperature (T = 273 + t°).

In the case when the weight concentration Q (g/cm^3), radius r (cm) and density ρ (g/cm^3) are known for the particles of a monodispersed aerosol, the particle concentration n_0 (cm^3) can be determined by the formula (Ye. P. Mednikov, 1963):

$$n_0 = \frac{Q \cdot 10^{-6}}{\frac{4}{3}\pi r^3 \rho} = 0{,}24 \cdot 10^{-6} \cdot \frac{Q}{r^3 \rho}. \qquad (1.6)$$

The degree of lowering of particle concentration for an aerosol due to coagulation can be seen on the following example: we will assume that at a temperature of 20° (T = 293°) a weight concentration of a monodispersed aerosol of 10 g/m^3 is created. The radius of the particles is $2 \cdot 10^{-5}$ cm and the density is 1 g/cm^3. Determine the change in particle concentration of the aerosol after 10 and 100 seconds.

The following particle concentration corresponds to a weight concentration of 10 g/m^3 (per formula 1.6):

$$n_0 = 0{,}24 \cdot 10^{-6} \cdot \frac{10}{(2 \cdot 10^{-5})^3 \cdot 1} \approx 3 \cdot 10^9 \text{ cm}^{-3}.$$

For the given conditions the coagulation constant will be

$$K_0 = \frac{4 \cdot 1{,}38 \cdot 10^{-16} \cdot 293}{3 \cdot 18{,}3 - 10^{-3}} \left(1 + 1{,}246 \frac{0{,}653 \cdot 10^{-5}}{2 \cdot 10^{-5}}\right) = 0{,}42 \cdot 10^{-9} \text{ cm}^3/\text{s}.$$

Particle concentrations are as follows:

after 10 seconds

$$n_{10} = \frac{3 \cdot 10^3}{1 + 0{,}42 \cdot 10^{-9} \cdot 3 \cdot 10^3 \cdot 10} \approx 1 \cdot 10^3 \text{ см}^{-3};$$

after 100 seconds

$$n_{100} = \frac{3 \cdot 10^3}{1 + 0{,}42 \cdot 10^{-9} \cdot 3 \cdot 10^3 \cdot 100} \approx 2 \cdot 10^3 \text{ см}^{-3}.$$

Assuming that the particles of the aerosol are uniformly distributed in a unit volume it is possible to determine the radius of the aerosol particles after their adhesion to one another, considering that the aerosol remains monodispersed in the course of coagulation (Ye. P. Mednikov, 1963).

$$r = r_0 \sqrt[3]{\frac{n_0}{n}}. \qquad (1.7)$$

In our example after 100 seconds the radius of the particles will equal

$$r = 2 \cdot 10^{-4} \sqrt[3]{\frac{3 \cdot 10^3}{2 \cdot 10^3}} \approx 5 \cdot 10^{-4} \text{ см}.$$

The rate of coagulation of aerosol particles is influenced by various factors: the degree of dispersion of the phase, the degree of uniformity of the particles, and the shape and electrical state of the particles. Of the enumerated factors which determine the coagulation rate the most important are the shape and size of the particles and the degree of their uniformity. Other conditions being equal, the higher the degree of dispersion of the particles the more rapidly coagulation will occur. Smoke coagulates more slowly, the more uniform it is - i.e., the

closer it approximates an isodispersed system. Smokes which possess a clearly expressed polydispersed nature coagulate rapidly, with the larger particles serving, as it were, as centers of coagulation for the smaller ones.

Coagulation of particles of nonspherical shape occurs more rapidly than that of spherical particles. An electrical charge of identical sign hinders coagulation of the particles; however, in many cases induction forces can convert repulsion between the particles into attraction. A charge of different sign on the particles facilitates coagulation. A moderate bipolar charge has no influence on coagulation rate and intensive charging of an aerosol is the only factor which can somewhat lower its stability.

The stability of aerosol systems whose dispersed phase consists of liquid droplets depends on evaporation and condensation. The content of vapors of the substances of the dispersed phase in the dispersion medium corresponds to the pressure of these vapors at the given values of temperature and pressure.

In an aerosol cloud of constant volume a state of mobile equilibrium is very quickly established between the dispersed phase and the dispersion medium; this, however, is disturbed with a change in temperature and pressure - for practical purposes, mainly with a change in temperature. An increase in temperature causes evaporation, while a lowering of temperature leds to condensation. The stability of the aerosol system is also influenced by the size and shape of the particles. With a reduction in particle size the vapor pressure on the surface of the particles grows; the vapor pressure is greater on a convex surface and lower on a concave surface than on a flat one.

In connection with the fact that the vapor pressure on the surface of small particles exceeds that of the dispersion medium,

processes of evaporation from the surface of small particles predominate over processes of condensation; this causes a reduction in the size of the small particles. With respect to large particles the reverse is true. In connection with the fact that the vapor pressure on the surface of such particles remains stable, not exceeding the average vapor pressure of the dispersed phase, processes of evaporation from the surfaces of such [large] particles lag behind condensation processes in magnitude; this results in the large particles becoming even larger. The described phenomenon of growth in a polydispersed system of large particles due to the reduction in the size of the small ones is called isothermic distillation. In the end all of the small particles are evaporated and the enlarged particles precipitate out into a deposit.

Under natural conditions the evaporation of droplets of water can be retarded, since air will frequently contain vapors of various organic substances whose molecules, being absorbed onto the water droplets, create an extremely thin film which is impermeable to the vapors. Such a phenomenon is noted in the presence of protein substances.

All of the aerosol properties examined above (sedimentation, coagulation, evaporation) are of great theoretical and practical significance for investigation of systems of bacterial aerosols. However, the physiochemical and colloidal properties of aerosols and methods of studying them cannot be mechanically transferred over to bacterial aerosols. Study of the physical and physico-chemical properties of aerosols must be carried out with consideration of their biological peculiarities.

2. Bacterial Aerosols

Bacterial aerosols is the term applied to aerodispersed systems in which the dispersed phase is made up of microorganisms

suspended in air, either existing independently or located in droplets of moisture and on flecks of dust.

Thus bacterial aerosols consist of a combination of either a gaseous medium and a solid phase ("droplet nuclei," bacteria and dust) or a gaseous medium and a liquid phase (bacterial drops). Bacterial aerosols make up a system whose behavior is determined by both physical and biological laws. The behavior of the aerosol as a physical system depends primarily on the size of its particles and the kinetics of the gas phase.

Bacterial aerosols are subject to the fundamental laws inherent to aerosols proper, making up a colloidal system which possess biological properties and in which the dispersed phase is living material - a bacterial cell or the liquid and solid substances with which these cells are connected - and a dispersion medium - air.

Bacterial aerosols can both be formed under natural conditions and can be prepared artificially. Dispersion of dust or liquid containing bacteria gives rise to natural bacterial aerosols. If a liquid bacterial culture is atomized, bacterial fogs will be formed; in the case of a dry culture the product is bacterial dusts. With respect to the mechanism of formation, bacterial aerosols will always be of the dispersion type.

Dispersion can occur due to high speed of air flows or by breaking up of liquids into droplets.

Natural bacterial aerosols arise indoors and in the open atmosphere due to physiological acts of humans (sneezing, coughing) and animals (snorting) and also under conditions connected with events which raise infected dust into the air.

Artificial bacterial aerosols are formed as a result of dispersion of bacterial emulsions or bacterial dust.

Natural and artificial bacterial aerosols can be found in different phases: a drop phase, a phase of dessicated dehydrated bacterial drops - the drop-nucleus phase - and in a dust phase. The drop phase consists of bacterial cells surrounded by a water-salt shell; protein, mucin, and other substances can enter the cell. The outer shell is subjected to rapid evaporation, leading the drop phase to lose its properties. The bacterial drops are converted into the phase of dried bacterial drops - "nucleoli." "Nucleoli" may be present in structures which consist of bacterial cells stripped of the layer of free water but retaining absorbed water, along with chemically bound water on the surface and free water inside the cell. Dry salt, protein, and other substances present in the cell prior to evaporation of the water may be found on the surface of the bacterial cells. In the phase of dry bacterial drops the particles have the smallest size; they are easily moved by air currents and remain in the air for a long period.

Consequently, the phase of dry bacterial drops is the most stable of the three phases in terms of colloidal and chemical properties. Subsequently the bacterial particles in the phase of dried bacterial drops are deposited on particles of dust in the air or on the floor or furniture. Bacterial dust is easily dispersed by even very small air currents. The time during which the dust phase of a bacterial aerosol remains in the air is dependent upon the size of the dust particles.

Particle size in bacterial aerosols varies in a wide range - from 1 mm down to 0.01 μm. A change in the size of aerosol particles due to one factor or another is accompanied by a quantitative change in the properties of the aerosol. The lower boundary of dimensions of bacterial aerosols may lie

within the limits of these dimensions of the bacterial cell. A further reduction in their size is limited both by the size of the bacterial cell and by the possibility of disintegration of suspensions and powders.

A double method of propagation of bacterial drops by air has been established. The so-called dynamic projection of drops in air occurs through kinetic energy obtained by the drops at the moment of ejection during a spasm of the muscles of the respiratory tract (acts of sneezing, coughing, etc.). During sneezing and coughing the maximum velocity of the air flow in the bronchi can reach 300 m/s. According to data from Duquid (1946), while the initial velocity of a jet of air in loud conversation amounts to 16 m/s and that during sneezing is 46 m/s, during a cough it exceeds 100 m/s. Along with horizontal motion of the droplets they are shifted rapidly downward by the force of gravity. The distance of dynamic projection depends on the size of the drops and their initial velocity. It has been established that with an initial velocity of 46 m/s the radius of propagation comprises 11 meters for 1-mm drops and 1.1 m for drops 0.1 mm in diameter; a radius for 0.01 mm droplets is 0.13 m. Under favorable conditions and with an initial velocity of 46 m/s the radius of scattering for 1-mm drops can reach 45 meters. Consequently, drops of large dimensions can be scattered over great distances during dynamic projection.

Another method of propagation of bacterial droplets is their transfer by a flow of air. The finest droplets, 0.1 mm and less in diameter, and also droplets possessing "intrinsic" kinetic energy can be spread by this method.

A number of authors present data which indicate extremely significant bacterial contamination of the air directly by acts of sneezing, coughing, etc. In the case of a vigorous sneeze up to 40,000 droplets containing bacteria, mainly from

the "nasopharyngeal microorganism" group, can be detected in air (Jennison, 1941; Bourdillion, 1948; S. S. Rechmenskiy, 1951). Droplet size varies from 1 to 1000 μm and more, with 2/3 of the droplets having dimensions of less than 100 μm (Mitman, 1945; Jennison, 1942; Duquid, 1946; Wells, 1955).

The French investigator Trillat (1938) found droplets 1 μm in size. These droplets are similar in properties to inorganic aerosols found in the air.

A smaller quantity of droplets is liberated during a cough; their size varies within the limits 1-2000 μm, with the size of droplets in the main mass falling in the 2-40 μm limits.

Bacterial droplets can also be emitted during loud conversation (P. N. Lashchenkov, 1899). Duquid (1946) found that during conversation at different levels up to 210 bacterial droplets around 100 μm in diameter are released.

Green, Vesley (1962) made a careful study of the quantity of bacteria emitted from the nasopharynx of a human into the surrounding air during conversation. The study was carried out in an experimental chamber and in an open space, where the subject repeated one and the same phrase every ten seconds for the space of one minute. The air was checked by means of an Andersen sampler (Andersen, 1958) and by the method of deposition on Petri dishes. Blood agar with 5% defibrinated human blood was used as the nutrient medium. During conversation more than 5000 bacteria were trapped from a calculated 100 *l* of air. The authors detected particles less than 4 μm in diameter. These studies substantially supplemented observations by Jennison (1942), carried out by means of high-speed photography.

The time during which the finest droplets were retained in the suspended state was determined by the speed of motion of the air.

It has been noted by a number of authors that large drops will, for the most part, drop out in the first minutes after they are emitted into the air, while finer droplets may remain in the suspended state for several hours (L. M. Gorovits, 1916; S. S. Rechmenskiy, 1951, and others). Rechmenskiy showed experimentally that droplets more than 100 μm in diameter remained in the suspended state only when the speed of air is at least 3 m/s; in this case they are scattered over a radius of no more than 1 meter.

With respect to the phase of dried bacterial drops "nucleoli" information is extremely limited. According to data from Mitman (1945), dried bacterial drops have dimensions of less 5 μm. Duquid (1945) considers that the bulk of these particles will fall within the limits 1-4 μm.

According to data from a number of observations the nature of the drops emitted during acts of sneezing and coughing is extremely nonuniform. Thus, a sneeze will emit drops obtained from the saliva and from the mucous membranes of the nose and respiratory tract. The content of bacteria in the droplets varies widely. Large drops and filaments of mucous may contain many microorganisms, while small drops may have none at all or contain only 1-2 microorganisms. Thus a sneeze releases into the air mainly microorganisms which are vegetative in the saliva (Streptococcus salivarius and others), while a cough will liberate a large quantity of β-hemolytic streptococci which live on the tonsils and in the pharynx.

The concept of "physical and biological decay" is characteristic for the system of a bacterial aerosol. By the concept "physical decay" of the aerosol we understand deposition, washing away by deposits, adhesion of particles to surfaces, etc. The action of these factors reduces both the number of particles suspended in the air and their concentration in a unit of volume.

Factors which cause biological decay of an aerosol system include the action on microorganisms of solar radiation, and the temperature and humidity of the air, as well as the time during which the particles remain in the environment.

The stability of the dispersed phase of a bacterial aerosol depends on the size and degree of isodispersion of the suspended particles, their electrical charge, the humidity of the air, and also the ability of the particles to coagulate.

Three phases are singled out in a bacterial aerosol with respect to sedimentation rate: large-nuclear - particles more than 100 μm in diameter with a rate of particle deposition greater than 0.3 m/s (this is a low-stable phase); fine-nuclear - particles less than 10 μm in diameter, settling rate less than 0.3 m/s. Thanks to the high specific surface and low weight the drops are held in the air for a prolonged period and dry out before they settle. The third phase is that of bacterial dust - coarsely and finely dispersed phases which have been dried out and transformed into bacterial dust; the latter is easily entrained by air currents and gradually settles out once again. The rate of particle transfer is 0.3 m/s-0.3 m/min. Particle size is 1-100 μm.

The sedimentation rate for particles of a bacterial aerosol obeys laws inherent to ordinary aerosols and can be calculated by the Stokes (1.1) and Stokes-Cunningham (1.3) formulas.

Table 3 (from L. M. Levin, 1961) gives data on the rate of settling in unmoving air for drops of water vapor as a function of droplet size.

The process of coagulation of particles of a bacterial aerosol can be approximately expressed by the Smolukhovskiy equation (1.5), while the change in dimensions of liquid particles

in the process of coagulation can be determined from equation (1.7).

Table 3

Drop diameter (μm)	Settling rate (cm/s)
1	0.003
5	0.076
10	0.3
50	0.6
100	30

The fate of droplets after they have been emitted into the air depends primarily on their size. Large drops and filaments of mucous settle rapidly, dry out, and become mixed with particles of dust.

The dust phase of the bacterial aerosol predominates in the air of living quarters and in areas intended to contain animals. Fine droplets, gradually settling, are rapidly reduced in size due to loss of water by evaporation. The rate of evaporation depends primarily on differences between the pressure of water vapor in the atmosphere and in the drop and also on the shape of the drop. Since the vapor pressure on the surface of a drop containing dissolved material is less than the pressure in a drop of water, it is evaporated more slowly than a drop of water in the same atmosphere. With an increase in the concentration of dissolved substances the rate of evaporation is essentially reduced. The size of the droplet is reduced until the vapor pressure inside the drop becomes equal to the atmospheric vapor pressure. With evaporation of water the weight of the

droplet is reduced and "droplet nuclei," which settle slowly, can remain in the air for a prolonged period. Thus, with atmospheric humidity within the limits of 50% droplets less than 100 um in diameter are almost completely evaporated at a distance of less than 30.5 cm from the point of formation. With atmospheric humidity of 90% the evaporation of the same droplets occurs at a distance somewhat greater than 304.8 cm.

The question of the reasons for the accelerated death of microorganisms located in particles of a bacterial aerosol at different states of the atmospheric medium has as yet been inadequately studied. Apparently the main condition which leads to death of microbe cells in air is loss of water by the aerosol drop and by the microorganisms. It is known that a bacterial cell contains up to 80-90% water, while spores of the bacilli contain as little as 40%. Besides the free water in the biological materials, even in particles of bacterial aerosols there is always a certain content of water which is absorption-bound to substances which possess hydrophilic properties. Bound water possess certain characteristic features which distinguish it from free water. Thus, bound water of protein solutions has higher density as compared with free water. Bound water is not a solvent. Removal of bound water requires enormously greater energy than removal of free water; in connection with this fact, during the ordinary process of evaporation in the atmosphere bacterial droplets will for the most part lose only free water.

Along with substances of a complex organic nature, the composition of bacterial cells includes salts in solution in the form of electrolytes. The normal vital activity of the cells requires an optimum concentration of these salts. When the cell is dried out as a result of a reduction in the quantity of water, the concentration of the electrolyte salts is increased. This leads to irreversible changes in the cell - denaturation of the

bacterial protein (R. Greaves, 1956; B. I. Blankov and D. L. Klebanov, 1961). Drying also leads to concentration of the protein molecules and to their aggregation, which causes destruction of the cell.

Rosebury (1947) also considers that evaporation from cells located in air in the state of a droplet phase aerosol is a cause of their death. These considerations were confirmed by experiments with Bacillus prodigiosus (Serratia marcescens) suspended in distilled water and in a solution of gelatin. The index of [microorganism] recovery averaged 0.9% for water and 14.1% for the gelatin solution. Similar data were obtained with other cultures of microorganisms.

Observations indicated that the die-off of microorganisms is more intensive in coarsely dispersed aerosols as compared with finely dispersed systems, since in the latter in the course of dehydration the microorganisms are subjected to the action of lower concentrations of substances which are harmful to the cell.

Two periods are distinguished in the death of the microorganisms occurring in air in the aerosol state. The process which causes death of microorganisms in the aerosol and which continues in the first minutes of the aerosol's existence is apparently connected with changes in the osmotic and chemical activity of the cell - i.e., it is a result of drying. The second process is more prolonged and is connected with destruction of microorganisms in dried preparations; in the opinion of Perry and Narle (1954) it is dependent on slow oxidation.

Webb (1959, 1960) noted that in an aerosol (bacterial cultures on distilled water) over a period of 5 hours there first occurred rapid death of the cells, in the first seconds after atomization, with a slower stage of death being observed

in the subsequent period. It was established that the death rate is directly connected with the values of temperature and relative humidity of the air.

The detailed studies of the effect of humidity and temperature of the air on viability of microorganisms in the aerosol state were carried out by Dunklin, Puck (1948), Brown (1953, 1954), Perry, Marle (1954), V. S. Yarnykh (1962), and others.

Dunklin and Puck found in carrying out experiments with pneumococci in a droplet-phase aerosol state that with variation in relative humidity of the air from 3 to 80% and at temperatures of 14, 22, and 33° the most intensive die-off of the microorganisms is found in a narrow range of humidity variation, in limits around 50%. At lower or higher indices the pneumococci survive for a substantially longer time. The same data were obtained in experiments with staphylococci and streptococci.

In experiments with intestinal baccili (E. coli) and staphylococci (Staph. albus), Williamson and Gotaes (1949) arrived at the conclusion that the most favorable conditions for retention of vital activity by these microorganisms are created at low values of relative humidity of the air. This was also confirmed in works by the following authors: Edward, Elford, Laidlaw (1943), and also Loosli, Lemon, Robertson, Appel (1943), who showed that the influenza virus dies out substantially more rapidly when dispersed in moist air than in dry.

The vital activity of bacterial cells of the Serratia marcescens and white staphylococcus (Staph. albus) in the aerosol state was studied by V. V. Vladavets (1968). The Serratia was atomized in a mixture with physiological solution. The change in the concentration of viable cells in air was determined by the method of settling and by means of Rechmenskiy bacterial trap. Vladavets noted extremely rapid die-off of

the Serratio baccili in air at low levels of relative humidity. With air humidity of 55-60% the viability of the cells grew sharply. The results of the studies are presented in Table 4.

Table 4. Effect of relative humidity of the air on the viability of Serratio marcescens in an aerosol with 10-minute exposure (per V. V. Vlodavets, 1964).

Relative humidity of the air (%)	Temperature (C)	Number of colonies grown in Petri dishes						
		Immediately after atomization	After various periods of time following termination of atomization					
			10 min	20 min	30 min	1 h	2 h	4 h
20	20	30	0	0	0	0	0	0
30	21,5	1054	442	6[illegible]	19	1	0	0
41	19	588	312	165	165	24	0	0
57	18	2200	1444	1100	970	220	1	0
68	19	2196	1540	877	311	103	1	1
75	19	7454	4728	2944	2014	523	55	4
85	18	10432	7444	5600	4485	1020	220	4

At the same time, a change in humidity has no essential influence on the concentration of viable staphylococci. The concentration of viable staphylococci was retained somewhat longer at low levels of humidity and dropped more rapidly at humidity above 90%.

The temperature of the air has a major influence on the viability of microorganisms in the state of an aerosol containing liquid droplets. At present we can consider it to be established that at different temperatures the humidity of the air has extremely different effects on viability.

Brown (1953) revealed essential differences between viability indices of bacteria in an aerosol at 0° and at 10°. While the lowest death index for all types of studied bacteria (Escherichia coli, Achromobacter, Pseudomonas) at a temperature of 0° was recorded with 70% relative humidity, the maximum die-off of Achromobacter was noted at 10°. At 10° there was a sharp increase in the death rate of E. coli and Pseudomonas recorded with a reduction in relative humidity below 60%.

Kethley, Fincher, and Cown (1957), investigating the effect of temperature in the range from -32 to +40° on the viability of Serratio marcescens in an aerosol also established that humidity has a different effect on viability of the bacteria in the aerosol at different temperatures. In the opinion of these authors, in hot wheather (with the exception of extremely dry air) the possibility of dissemination of aerogenic infections is minimal, owing to the insignificant viability of microorganisms in an aerosol. With a more moderate temperature regime the favorable conditions for microorganisms are created at high levels of relative humidity. In the conditions of low temperatures bacteria are stable in an aerosol over a broad range of relative humidity values.

V. S. Yarnykh (1962) reports on the effect of temperature and humidity of the air on P. avium and certain other types of microorganisms suspended in the air. The most favorable temperature for retention of a bouillon culture of Pasteurella is 12-15°. Up to 28% viable microorganisms remain in the air. At 21-22°, 60 minutes after atomization, only 9.4% of the microbes can be separated from the air. At a temperature of 28-30° virtually all Pasteurella and intestinal bacilli die out after 10 minutes in air. Staphylococcus aureus turned out to be the most stable. Changes in air temperature are interconnected with relative humidity. Thus, within the limits 50-90% humidity, at a temperature below 16° the stability of Pasteurella was increased while at 17-25°, on the other hand, acceleration in death of Pasteurella is noted.

The stability of causative agents of infections in the environment and retention of properties by them depend on whether or not the microorganisms are connected with droplets of fluid containing any sort of nutrient or protective substances or whether the bacteria are located in the air in "pure form." Naturally, microorganisms suspended in the air

"without protection" are enormously less resistant to the action of environmental factors - i.e., the phenomenon of biological decay of the aerosol system will be manifested more sharply in this case.

The medium of a suspension usually includes substances which lower the death rate of microorganisms in the aerosol state. Such protective media most frequently contain colloids. Heller (1941) showed that the protective properties of the medium are improved when its hydrophilic qualities are increased. S. S. Rechmenskiy (1951) used meat-peptone bouillon as the protective medium. It was found that particles of aerosol containing the bouillon were more viable than an aerosol of the same microbe suspension based on distilled water.

Colloidal substances located in drops of an aerosol exert a protective action on microbe cells, protecting them from death, and protecting the droplets from evaporation. Under the influence of colloidal substances the droplets of an aerosol take on colloidal-chemical properties facilitating their stability in air. The maximum die-off of microorganisms occurs in the absence of protein protection on them in the aerosol state.

Webb (1959, 1960) made a detailed investigation of the various factors which influence bacterial aerosols. He studied the role and influence of distilled water (used as a component part for the preparation of a microbe suspension) and also various chemical additives. He also clarified the role of bound water on the change in the structure of a protein in the process of survival (in the aerosol state) of Serratio marcescens , intestinal bacilli (E. coli), Staph. albus , and of the Bac. subtilis . The average size of the aerosol particles was 5 μm. Webb showed that the addition to the initial suspension of amino acids and of substances which shorten the protein chain, as well as sugars and polyhydrohydroxycyclohexanes, increases the viability of the cells in the aerosol state.

In Webb's opinion, the increased viability of cells during drying in air in the aerosol state is connected with the presence of amino groups or secondary alcohol groups. The hydroxyl group is toxic in the benzene ring and possesses protective properties in the pyrimidine ring. It is proposed that these compounds replace water in the protein structure during drying of the cell, which leds to retention of the structure of the proteins. Of primary importance in injury to cells during drying in the aerosol state are the cell "shells," which are destroyed and lead to loss of differentiation within the cell.

Depending upon the stability of the microorganisms and the effect of environmental conditions, in certain cases biological destruction of a system of a bacterial aerosol can set in earlier than its physical decay, so that particles of the aerosol will contain dead microbe cells. In other cases the microorganisms settle out in a viable state and, forming an aerogel, give rise to the appearance of a secondary bacterial aerosol, since the particles are capable of reentering the suspended state under the effect of environmental factors.

The infective action of bacterial particles in the aerosol state depends upon the type of microorganisms, their concentration in the air, and also on the degree of dispersion of the system.

Contamination can occur through exposed portions of the body when there is damage to the skin, through the gastrointestinal tract, through the conjunctiva of the eye, and, in particular, through the respiratory organs. The latter is the specific and most important route of contamination in the case of airborne infection. The nature and particular features of the effect of a bacterial aerosol on the organism are determined by the following factors: the biological and physicochemical nature of the aerosol, the quantity of biologically active particles retained in the organism -

i.e., the dose of the infectious agent, the initial distribution of these particles in the organism, and the subsequent fate of the retained particles (elimination, redistribution, resorption, etc.).

The quantity of microbes which enter the organism through the respiratory tract from a bacterial aerosol is determined by the concentration of the aerosol, exposure (length of time during which the aerosol is breathed), the volume of pulmonary ventilation, and retention of the aerosol in the pulmonary tracts.

The aspiration dose of the infectious agent can be calculated by the formula

$$D = c \cdot t \cdot v \cdot R, \qquad (1.8)$$

where D is the dose of the infectious agent; c is the concentration of the biologically active microbes; t is exposure; v is the volume of pulmonary ventilation; and R is the factor of retention of aerosols in the lungs.

The dose of the infectious agent is of decisive significance in the development of a disease. As studies by Rosebury (1947) showed, the aspiration infective dose and the lethal doses differ in different diseases and exceed the corresponding doses during subcutaneous and other forms of contamination.

The degree of retention of the aerosol in the respiratory organs will to a significant degree determine the dose of the infectious agent obtained by the organism during exposure. Deposit and retention of aerosol particles in the respiratory tracts is caused by gravity, inertia, Brownian motion, the degree of dispersion and electrical charge of the particles, etc. Thus, according to data from Rooks (1939, 1941), 80-90% of large

bacterial drops are retained in the nasal cavity during respiration.

N. V. Tatarskiy (1951), I. I. Yelkin, and S. I. Eydel'shteyn (1952) also consider that the depth of penetration of particles of a bacterial aerosol depends primarily on their size. In fact, all particles 10 μm in diameter and approximately 50% of particles 1-5 μm in diameter are retained in the nasopharynx (Sawyer, 1963).

These data indicate that the greatest danger in aerogenic contamination is from aerosols made up of individual bacterial cells. This consideration found confirmation in the works of Druett, Henderson, Packman, Peacock (1953) with contamination of guinea pigs and monkeys with anthrax. Aerosols containing individual anthrax spores turned out to be much more infectious than larger particles. In experiments with Br. suis and P. pestis (1956), it was shown that an aerosol with a particle size of 12 μm is 600 times less infectious than an aerosol with particles containing individual microbe cells.

Goodlow, Leonard (1961) reported on the infection of guinea pigs and monkeys with the tularemia agent as a function of the magnitude of particles of a bacterial aerosol penetrating the respiratory tract. Thus, for particles 1 μm in diameter the LD_{50} for guinea pigs was 3 cells and that for monkeys was 17 cells; for particle 7 μm in diameter the LD_{50} was 6500 and 240 cells, respectively, while for 22 μm particles the values were 170,000 and 3000 cells.

A similar dependence of infectiousness on aerosol particle size was detected for anthrax, brucellosis, virus equine encephalitis, and other diseases.

According to data from Hatch (1961), for the most part aerosol particles 10 μm in diameter and larger are deposited

in the respiratory tract. The percentage of deposition of particles less than 5 μm in diameter amounts to only 20-30%, while for those with a diameter less than 0.50-0.25 μm, the value is 60%.

Altshuler also studied the question of retention of microorganisms on droplets of an aerosol in the respiratory tracts. Data from his study are presented in Table 5.

Table 5. Retention of microorganisms in the respiratory organs as a function of the degree of dispersion of aerosol particles (Altshuler, 1935, 1957).

Particle size in microns (d)	10 and more	5-10	3.2	0.8-1.6	0.4	0.22
Percentage retention	100	80-100	61-80	19-48	17-33	19-36

The largest particles, subjected to the action of gravity and inertia, settled out in the upper respiratory tracts. With a reduction in the degree of dispersion the influence of the force of gravity and inertia is weakened and the particles are retained to a smaller degree, allowing them to penetrate into the deeper areas of the respiratory tract. Particles 0.3-0.4 μm in size have negligible weight and inertia during Brownian motion, which leds to an extremely small percentage retention of these fractions in the respiratory tracts. With a further reduction in particle size the effect of gravity and inertia is reduced and at the same time there is a substantial growth in Brownian motion, which once again leads to an increasing degree of retention of the aerosol particles.

Generalization of results from numerous studies on the distribution of aerosol particles in the respiratory organs as a function of particle size allows us to conclude that particles more than 15 μm in diameter are retained predominantly in the upper respiratory areas (nose, mouth, nasopharynx), particles 30-50 μm in size may penetrate to the trachea, while particles 10-30 μm in size do not reach further than the bronchi. Aerosols 3-10 μm in size penetrate to the bronchioles, while 1-3 μm and smaller particles may reach the alveoli (Sawyer, 1963).

However, the literature contains reports indicating that even larger particles, reaching 5-13 and even 80 μm in size, have been detected in the alveoli (Wotkins-Pitsford, Moizy, 1916; Faber, 1936; Bedford, 1950). If we consider that the diameter of the opening to an alveolus comprises 70-100 μm, the penetration into alveoli of particles of relatively great size can theoretically be recognized as completely probable.

Of substantial significance also are data on the weight distribution of polydispersed aerosols in the respiratory system. Abramson (1950), studying penetration of powdered crystalline penecillin with a degree of dispersion of 0.65 to 58 μm into the lungs and its distribution in the respiratory system of animals, found the following weight distribution of the particles: the trachea contains 45.15%, the main bronchi contain 43.15%, the alveolar paths contain 5.91%, and the alveoli contain 0.65%.

As is evident from the above data, with a polydispersed composition of the aerosol the main mass of particles is retained in the trachea and the bronchi. Consequently, during the primary distribution of aerosol in the organism - the respiratory organs - the relatively large particles are retained in the upper respiratory tracts, with penetration to the alveoli only of particles for the most part no larger than 3-5 μm.

The ability of aerosol particles to be retained in the respiratory tracts is essentially influenced by their electrical charge. Particles are attracted to the walls of the respiratory tract by induction forces. According to data from I. I. Livshits, Ye. T. Lykhina, and G. S. Erenburg (1948), during inhalation 34% of the uncharged particles and 66% of the charged particles of aluminum dust were deposited.

The subsequent fate of bacterial particles entering the respiratory tract is determined mainly by biological laws, with the point of deposition of particles being one of the main factors determining the outcome of the interaction between the macroorganism and the microorganism. During evaluation of the biological effect of a bacterial aerosol on the organism, the virulence of the microorganism is also of substantial significance; this factor can determine the nature of the infection process and the fate of the microbes introduced into the organism.

Particles of a bacterial aerosol are also characterized by certain common laws typical for indifferent particles. Thus, particles may be ejected by the organism into the ambient medium, they may reach the gastrointestinal tract, and they may be subjected to resorption. For the most part the particles ejected from the organism are the coarsely dispersed particles which settle in the initial period of penetration into the upper respiratory areas. As indicated by the studies of Langmuir and Andrews (1952), under the action of the ciliary epithelium particles are moved out together with secretions of the nose and nasopharynx during coughing, sneezing, etc. In this case particles 10 μm in diameter are 100% removed from the respiratory tracts, while 80% of the 5-μm particles are removed; for practical purposes particles 1-2 μm in diameter are difficult to eliminate.

If we assume that the length of the respiratory tract comprises 40 cm and that the rate of outward travel of a particle

is 15 mm/min, then it is obvious that large particles can be retained in the human respiratory tract for no more than 30 minutes (Thompson, 1959). As regards small and extremely fine particles, as indicated by the observations of a number of authors, phagocytosis of these bacterial particles by leucocytes and histiocytes is possible, followed by their removal from the organism along the bronchi with the flow of air (Harper, 1955; Ross, 1957; Dannenburg, Scott, 1958).

Resorption of particles of a bacterial aerosol can occur over the entire surface of the respiratory tract. The absorbing capacity of the respiratory apparatus is very great; this depends on the particular features of its mucous linings and also on its enormous surface. It has been established that the intensity of absorption differs in different sections of the respiratory tract. Resorption occurs more energetically through mucous membranes covered with siliary epithelium than through flat membranes (mouth, pharynx, esophagus, entrance to the larynx). The maximum intensity of absorption is observed in the lungs.

Harper (1955), infecting guinea pigs with brucellosis through the air, established that multiplication of brucellae reaching the lungs occurred on the surface of the epithelium, from which they were then transported to the lymph nodes, leading to generalization of the infection.

The ease with which aerogenic contamination is accomplished is explained by the physiological peculiarities of the structure of the respiratory apparatus. The human lungs have a surface of about 200 m^2, while the lungs of large agricultural animals have surfaces of 50-300 m^2; large quantities of blood are in direct contact with the surfaces. Particles of a bacterial aerosol containing microorganisms and arriving on the surface of the respiratory bronchioles are not subjected to the action

of the ciliary mucous coating. At this point phagocytosis of the microorganism by neutrophils and macrophages can occur. If this does not happen a bronchial infection can arise, which is extended to the adjacent alveoli and parts of the lung. Such primary involvement of the bronchioles in the process can occur during contamination by the causative agents of tularemia or psittacosis (Sawyer, 1963).

Consequently, the introduction of microorganisms can occur over the entire entent of the respiratory tract, beginning with the mouth and ending at the alveoli.

The laws governing the behavior of bacterial aerosols, the viability of microorganisms located in the aerosol particles, and penetration into the respiratory organs of human or animals form the basis of the mechanism of the dissemination of airborne infections. They must be taken into account during the development of measures to combat infections carried on airborne droplets.

3. Brief Epidemiological and Epizootological Characteristics of Bacterial Aerosols

Epidemiological public health and epizootological significance of air is determined primarily by its influence on the state of health of humans and also that of animals.

Even in the deepest antiquity infectious diseases striking humans and animals were recognised. Many scientists suspected "unhealthy" air to be the major cause of these diseases. Hippocrates gave great weight in his work to contamination of the air, considering "that all disease represents its flesh and its origin," and, supporting the theory of miasmas, divided diseases into those which threaten humans and those dangerous to animals.

In 1784 the Russian scientist Danilo Samoylovich (cited in V. I. Tets, 1958) proposed that infectious diseases might be

transmitted by means of microscopic essences and thus carried out the first investigations of air for bacterial contamination known in history.

Of essential significance for the subsequent study of the microbiological nature of infectious diseases with the works of Louis Pasteur, who in the XIX Century established the living nature of the causative agents of certain diseases of humans and animals. The works of Pasteur in the field of studying the causative agents of infectious diseases provided the impetus for the subsequent vast achievements in the field of bacteriology. In a period of 80-90 years after publication of the first works by Pasteur causative agents were discovered for the majority of infectious diseases and measures were developed to protect both humans and animals from them.

The concept of the existence of droplet-borne infection was put forward by F. F. Erisman (1872), who considered that purulent bodies or dense molecules of sputum liberated from sick persons and transported in the air might be carriers of infection and might contribute to the spread of certain epidemic diseases. However, at that time this proposal found no recognition.

At the end of the 1890's works appeared developed by G. Flyugge[1] (1897, 1904) which proves the possibility of direct infection of the air with extremely fine droplets of saliva and mucous, entering the air from the upper respiratory tract during acts of coughing, sneezing, and even during speech. However, he ascribed limited significance to the airborne method of transmission of infections and considered that the droplets of mucous and saliva, and even dry atomized infectious material, were

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[1]Exact spelling not found in available resources - Translator.

excessively large and settled out easily onto the floor. Their scatter radius does not exceed 2-3 m and therefore the danger of infection by them is not great.

Major credit in the business of the further study of the mechanism of propagation of airborne infections belongs to the Russian scientist P. N. Lashchenkov (1899, 1927), who as early as 1897 developed the Flyugge theory on droplet-borne infection and also arrived at the view, new in principle, of dissemination of infections of the respiratory tract with major significance being ascribed to droplet-borne infection. Prior to the discovery of P. N. Lashchenkov the words "dust" and "infection" were regarded as equivalent and interchangable. Lashchenkov proved experimentally that extremely fine contaminated droplets are of incomparably greater significance in the appearance of airborne infection than extremely fine dry dust. In this connection he wrote that "...in a great many diseases airborne infection by extremely fine droplets is of overriding significance. Such diseases can include all of those in which the causative agent of the infection is found in the mucous membrane of the oral cavity, the pharynx, nose, and also the bronchi, ... the danger of airborne infection is even greater in the case of influenza and other infectious catarrhal conditions of the nose, pharynx, and bronchi, and even more so with measles, whooping cough, consumption, pneumonia, diphtheria, leprosy, and smallpox."

The experiments upon which Lashchenkov based his conclusions consisted of a number of studies in which he determined the transfer of microbes by the air during low level and loud conversation, coughing, and sneezing. He established that during loud speech by a human with the oral cavity artificially contaminated with microbes (Serratio marcescens) the bacteria were disseminated over distances of 5-6 m. Lashchenkov considered that the negligibly small rate of air motion of 3-4 mm/s was

adequate for the transfer of droplets from place to place and for them to be retained in the air up to 5-6 hours, while large drops settled out rapidly. He did not deny the significance of dried dust in the dissemination and transmission of infectious diseases. In his opinion, the ability of one or another microorganism to undergo some degree of desiccation without losing its virulence is a condition for the formation of a dry infectious dust; the desiccation must be to that degree necessary for the infectious material to be capable of being broken up and transformed into dust. Summing up the ideas put forward by Lashchenkov, we can as a conclusion introduce his words: "It is clear and unquestionable that in all diseases whose causative agents are located on the mucous membrane of the mouth, pharynx, nose, and even the bronchi, airborne infection by direct spraying is a significant factor."

Various pathogenic microorganisms were detected in the air by Miquel (1889), A. D. Pavlovskiy (1885), N. Keldysh (1887), Moore (1893) and by a number of other investigators.

The causative agents of infectious diseases transmitted during infection of people and animals by the air are quite numerous. These include bacteria (streptococci, staphylococci, pneumococci, meningococci, and the agents of pertussis, diphtheria, tuberculosis, leprosy, ozeny, and scleroma), as well as viruses (the agents of measles, flu, psittacosis, ornithosis, chickenpox, and epidemic parotitis), along with rickettsia (agent of Q-fever and others). Thus it was shown that pathogenic microorganisms (in the state of droplet and dust phases of bacterial aerosols) are encountered in the air and are capable of infecting humans and animals.

The fate of a bacterial aerosol - to be exact, the survival of the microorganisms - depends both on factors conditioning the physical and biological conditions of breakdown of the system

and also on the biological features of the microorganisms themselves. Thus, the spore forms of microorganisms are easily retained in the aerosol, while the vegetative forms die out comparatively quickly.

A number of authors (Wells, Stone, et al. 1934) observed in room conditions retention of viability in the state of the fine-droplet phase of an artificial aerosol by several types of microorganisms. It was noted that the hay bacillus (Bac. subtilis) is retained in air for several days, while staphylococcus (Staph. aureus) survived for three days. The diphtheria bacillus and streptococci were detected in the air for up to two days. Microbes of the intestinal group lost their viability by the end of the first day. Consequently, the coccus group of microorganisms is more stable in air in the droplet phase of a bacterial aerosol than microbes of the intestinal group. It was proposed that the higher resistance of microbes - causative agents of infections of the respiratory tract - to environmental conditions is a consequence of their high degree of biological adaptability in the role of causative agents transmitted through the air. Similar adaptability could not be detected in the causative agents of the intestinal group of infections.

All droplets of a bacterial aerosol, regardless of their dimensions, finally settled out onto objects of the environment, dry up, and form bacterial dust. Numerous observations of pathogenic microbes in dust during human and animal illnesses form the basis in due time for many researchers to consider dust in dwelling areas to be one of the basic factors of the infection of humans as a result of contamination of the air during sweeping of the floor and general tidying up.

M. I. Maksimovich (1894) wrote: "Dust on the floors is one of the important agents of transmission of infections... the effect of raising dried dust is reflected extremely sharply

in the quantitative content of microbes in the air." Maksimovich found types of microorganisms detected in floor dust within the oral cavities of both healthy and sick individuals.

The primary forms encountered in bacterial dust are the saprophytic forms of the microbes, resistant to desiccation: sporiform bacilli and a variety of molds. The dust contains pathogenic mircoorganisms in a smaller quantity - pyrogenic cocci, the diphtheria bacillus, etc. Bacterial dust is of great significance in the dissemination of infections whose causative agents withstand desiccation.

Investigations carried out by A. I. Shafir (1951) on the retention of viability by microorganisms in conditions of dry air make it possible to consider that the tuberculosis bacillus, staphylococci, streptococci, pneumococci, the disentery bacillus, anthrax spores, the tetanus bacillus, and many forms of fungi are easily retained in dust and are disseminated together with the dust in a viable state. It was established that pyogenic virulent cocci are capable of remaining for a fairly long time in dust in living quarters, linen and clothing. The time during which viable streptococci exist in a room after the removal from it of a patient with a streptococcic infection varies within the limits 4-5 days.

S. I. Kudryavtsev (1957) found substantial quantities of both nonhemolytic and hemolytic Staphylococcus, Staphylococcus aureus, and also hemolytic streptococci in bedding and clothing. On the basis of comparative evaluation of the strains of staphylococci isolated from the air, bedding, and nasopharyngeal cavities of individuals staying in these rooms, it was established that all of the complex properties which characterize the biological activity of staphylococci were found in 9% of the isolated strains. The observations of Robertson (1953) attest to the

fact that hemolytic streptococci can retain viability in dust in a dark place for 5-8 months.

Generalized data from various authors on the survival rate of microorganisms on various environmental objects at temperatures of 10 to 20° have been presented by V. V. Skvortsov, V. S. Kiktenko, and V. D. Kucherenko (1960). Thus, the causative agent of plague remains viable and virulent in moist soil for up to 28 days; the tularemia agent survives for 75 days, that of brucellosis to 103 days, the cholera agent to 48 days, and the causative agent of meloidosis for up to 5 days. In dry soil the period of retention of viability is much less, comprising 1, 10, 25 and 4-5 days, respectively, for the microorganisms indicated above. On various fabrics the plague agent is retained up to 45 days, that of brucellosis for 30 days, cholera for 12 days, and the glanders agent for 14 days.

V. L. Omel'yanskiy (1941) notes that the plague bacillus retains viability under intensive desiccation for 8 days; the diphtheria bacillus survives up to 30 days, while staphylococci are viable for up to 70 days.

Certain viruses also were found to be extremely stable in air. According to data from S. M. Ostrovskaya, O. M. Chalkin and S. B. Olekhnovich (1938), the influenza virus loses its infectious capacity in 8 hours.

A. I. Shafir (1951) gives data on the ability of chickenpox and psittacosis viruses to easily withstand desiccation in dust and in the air.

After settling on the floor, bedding, and other objects the influenza virus can be retained on particles of dust which are once again raised into the air and inhaled by other people.

The influenza virus was successfully obtained from dried dust collected near an area where a polecat ill with influenza was found. A case is also described of infection of the investigator himself with influenza from the breath of a diseased skunk (Smith, 1963). From 1 to 10% of the emitted influenza virus is retained in dust on various objects in living quarters and withstands drying. Under these conditions the quantity of the virus is only slightly reduced in the first three days, with up to 10% of the virus being retained for a week and up to 1% for two weeks (Smith, 1963).

Interest in research on airborne microflora is growing every year. Characteristically, in these works the investigators connect the hygienic state of the air with its direct influence on humans and animals.

As is known, contamination through the air occupies a leading role in many diseases of humans and animals. In this respect especially favorable conditions develop in living quarters. While outdoor airs continuously mix over the surface of the earth, living quarters are merely ventilated. Consequently outdoor air is mixed with the internal and does not replace it entirely. Thus a mixture of fresh and room air is withdrawn from the area and a similar mixture remains within the dwelling. If there are no constant sources for arrival of microflora in the air of living quarters, the concentration of microorganisms in the room air is gradually reduced as a result of ventilation and settling, and also due to natural die-off.

The rate of removal or death of bacteria can be determined, according to Bourdillon and Lidwell (1948), by the expression

$$N = N_0 \cdot e^{-\kappa t}, \qquad (1.9)$$

where N_o is the quantity of microorganisms at the moment t = 0; k is the rate of removal of bacteria from the air by all factors acting over the entire time period studied.

The quantity K can take various specific values.

Thus, k_D is the death rate of living microbes; k_S is the rate of their removal by ventilation; and k_R is the rate of removal by settling.

Despite existing and continuously operating processes of air exchange and ventilation, a fairly large degree of bacterial contamination has been detected in living quarters as a result of a number of investigations. The degree of bacterial contamination of the air is directly dependent primarily on such factors as the density of population of the dwellings, the activities of the people, the degree of contamination with dust, the rate of air exchange, etc.

In this respect observations by Miquel (1883), Carnelly (1887), F. S. Epshteyn and E. G. Salamandra (1948), A. I. Shafir and P. A. Kouzov (1948), Swaebly (1952), S. I. Kudryevtsev (1957), Gregory (1961) and others are very indicative.

According to Miquel's data air in hospital rooms in Paris contained the following quantities of bacteria per m^3: in June up to 5110, in December up to 23,100 (an average of 11,000).

In 1887, Carnelly, using Hess tubes to study the air of schools and mills, noted a sharp increase in the total content of microorganisms in the presence of large numbers of people; simultaneously an increase in the degree of dust in the air was noted. When the air within the buildings remained quiet for a prolonged period the bacteria or the particles on which they are located settle out from the air more rapidly than mold fungi.

Maunsell, studying the bacterial level of air in a bedroom by means of a slotted collector during cleaning of the area, detected a sharp increase in the content of mold fungi in it.

A. I. Shafir and P. A. Kouzov, as well as S. I. Kudryavtsev, studied the change in the bacterial level of air in living quarters, in particular in hostels during periods of occupation and times of vacancy.

According to Kudryavtsev's data there is a direct dependence between the magnitude of bacterial level in the air of living quarters and the activity of the people occupying them. The greatest magnitude of the bacterial level in the air for sleeping quarters was observed, for example, in periods when people were awakening and rising from bed, when they were going to sleep, and especially during periods when the areas were being cleaned. The total quantity of microflora per 1 m^3 of air in studies with the Krotov apparatus and the Rechmenskiy siphon trap in these periods reached 12,000-25,250 microbe bodies, while in a period of relative calm there were 3600-5500 microbes per m^3. It was also determined that the presence of a large number of people in the area and, in particular, the presence of individuals with angina or catarrhs of the upper respiratory tracts will lead to contamination of the air with pathogenic microflora (β-hemolytic streptococci and hemolytic staphylococci) (Table 6).

In 1961 Gregory used a portable trap of his own design to study samples of airborne dust. The composition of the dust included small scales from the epidermal layer of human skin. Thus, prior to cleaning up of a room a few thousand of these potential bacterial carriers were detected per m^3 of air, while after the beds were made up the number grew to 390,000. Gregory considers that the larger portion of the bacteria existing in room air are fixed on these scales.

Table 6. Bacterial contamination of the air in sleeping quarters in a hostel in a 24-hour period (per S. I. Kudryavtsev, 1957).

Periods of observation of the area	Time	Quantity of microorganisms per m^3 of air			
		Total amount	Staphylococci albus	Hemolytic staphylococci	Hemolytic streptococci
No people present	9-12	1450	650	50	5
People present	21-22	5500	2250	250	50
Nocturnal sleep	4	4180	1200	220	25
Daytime rest	14-15	8500	3100	400	45
When people are rising from sleep and preparing to go to bed	7 and 24	25,250	11,130	540	75

Of essential significance are works concerned not only with studying the general level of bacteria in the air of living quarters, bu also dealing with the detection of various types of pathogenic agents of diseases in it, in particular microflora of the coccus type. Thus, S. S. Rechmenskiy (1944), F. S. Epshteyn and E. G. Salamandra (1948), N. N. Shastin (1954), S. I. Kudryavtsev (1957) detected α- and β-hemolytic streptococci in living areas and public buildings. Similar observations were made by O. P. Lebedeva (1953), A. S. Kaplan (1951), and others.

Data given below on infection of humans by diseases of the upper respiratory tract clearly indicate the major role played

by air as a means of transmitting the causative agents of infectious diseases.

Infectious diseases included in the group of upper respiratory infections are the most widespread. The wide dissemination of these infections is due to the ease with which the mechanism of transmission inherent to this group of diseases is realized. Numerous observers have also established that certain causative agents of infectious diseases whose transmission mechanisms include the absence of air under natural conditions can penetrate the respiratory tracts of humans and animals in the form of artificial bacterial aerosols and thus cause infection.

At present a large mass of material has been accumulated by accidental infection of humans and animals through the air. Thus, Feiner (1948) indicates that in the practical operations of American bacteriological laboratories cases have been noted of airborne infection (as the result of accidents) by the causative agents of anthrax (25 cases), brucellosis (17 cases), tularemia (7 cases), glanders (6 cases), and psittacosis (1 case).

Infection can occur when bacteria of melioidosis, plague, typhus and other microorganisms enter the air. In 1946 M. K. Krontovskaya, F. G. Krotkov, et al. published material on laboratory infection of humans by typhus through the air.

Under natural conditions the transmission of infection through the air is observed in the case of many infectious diseases of both birds and agricultural animals (contagious pleuropneumonia of horses, infections of the upper respiratory tracts of horses, hog cholera and influenza and grippe of hogs, tuberculosis of horned cattle, and other diseases).

The possibility of droplet-borne infection in the case of tuberculosis was proved by direct experiments by many investigators

using guinea pigs and calves, where individual tuberculosis bacilli were located in separate parts of the lungs after inhalation of contaminated material.

Infection with swine influenza under both artificial and natural conditions occurs only when the contaminated material enters the organism through the respiratory apparatus.

M. S. Gannushkin (1961) presents the case of transfer of infection (infected droplets) in epidemic pneumonia of horned cattle by air currents at a distance of 8-10 m, with total elimination of other methods of transmission.

Dust-borne infection can occur in animals in the case of anthrax and tuberculosis. Schwartz and Mathews (1954) showed the possibility of aerogenic infection of swine with hog cholera under production conditions.

The air is also one method for dissemination of animal viruses. Infection of sheep with sheep-pox has been described in the case when they were driven along a road over which animals ill with this disease had passed previously.

The virus of Newcastle disease or poultry fever has been isolated from quarters in which sick birds were held; air from contaminated quarters taken in amounts of 540 and 1080 ℓ contained the virus in concentrations adequate for infection of chick embryos.

Extremely fine bits of feathers and excrement from parrots infected with psittacosis and carried through the air can cause infection in both birds and humans (Smith, 1963).

The possibility of contamination of birds with plague through the air not only in artificially created virus aerosols,

but also in poultry yards infected with the disease has been demonstrated experimentally. Thus, P. N. Svintsov (1951) reported on such an experiment carried out in a poultry yard contaminated by fowl plague. In this poultry house cages with healthy chickens were suspended from the ceiling; the birds were infected with the plague through the air.

All other methods of transmission of the infection were excluded. P I. Pritulin presented interesting data in his works (1959), studying the pathogensis of parrot typhoid with various methods of infection - enteral, parenteral, and aerogenic. 329 animals were infected enterally, 263 parenterally, and 462 aerogenically. Infection was conducted with different types of paratyphoid bacteria: suispestifer, Gärtner, Breslau, and the causative agent of aborta ovis. Experiments showed that an especially rapid development of the infection process occurred as a result of aerogenic infection.

It has been proved experimentally that polio and influenza virus can be transmitted by susceptible animals through artificially contaminated air. The intensity of scattering of the infectious principle in the ambient medium through air frequently leads to rapid infection of all susceptible livestock.

M. S. Gannushkin (1961) considers that in aerogenic infections and "especially in the droplet method of transmission, if the incubation period is short, epizootic disease is disseminated very rapidly and covers all susceptible livestock, occurring in extremely short periods."

As a whole the problem of infections of the respiratory tract is far from satisfactory resolution. The enormous economic losses caused by "droplet" infections make it mandatory that workers in the antiepidemic front intensify the study of this question in all of its various aspects. There is no

question that studies directed toward investigation of the air as a factor in the transmission of infections in this group occupies a significant place in this plan.

CHAPTER II

GENERAL METHODS AND PRINCIPLES FOR DETERMINING CONCENTRATIONS AND DIMENSIONS OF AEROSOL PARTICLES

The determination of the quantity of particles for aerosols of organic and inorganic nature per unit volume of air is of vital significance in characterizing the aerosol. Calculation of the quantity of dust particles characterizes the dust content of the atmosphere, air in industrial areas, etc. Determination of the concentration of bacterial particles in the air makes it possible to evaluate the epidemiological situation and to determine the need for carrying out sanitation measures of one type or another.

The quantity of aerosol particles per unit volume of air depends on a variety of conditions: intensity of dispersion in condensation processes, leading to the appearance of dispersed phase of the aerosol; the physical and chemical nature of this phase; the age of the aerosol; atmospheric conditions, etc.

During determination of aerosol concentrations it is important to establish the method of expressing the quantitative characteristics of the content of aerosol particles per unit volume. Usually the calculated and weight concentration of aerosols are determined. The weight or gravimetric concentration is the weight of the aerosol particles contained in a unit volume of

medium. By the term calculated (particulate or konimetric concentration) we understand the number of aerosol particles in a unit volume of the medium. In sampling aerosols from a flow L. M. Levin (1961) singles out the concept of the flow concentration of an aerosol, in contrast to the concept of particulate concentration. Other authors base their work on the concept of particulate concentration alone.

In addition, in microbiology the concept of the cell concentration of an aerosol exists; this is defined as the number of cells per unit volume, (cells)/cm^3. Besides the practical determination of the particulate concentration of an aerosol, in research work the need may arise for tentative theoretical evaluation of this characteristic before the experiment is set up. Tentative calculation of cellular and particulate concentration can be carried out under a number of assumptions.

We will assume that within a closed volume (chamber) W(cm^3) it is necessary to create a particulate aerosol concentration (C[particles/cm^3]) or a cellular concentration (C_0[cells/cm^3]). We will assume that the aerosol generator used to disperse an initial suspension containing n_0 cells per cubic centimeter will create a monodispersed system in which all particles are virtually identical in size and which contains an identical number of cells. Then the number of aerosol particles obtained from A(cm^3) of the initial suspension will equal

$$n = \frac{A \cdot \Delta_1}{d^3}, \qquad (2.1)$$

where Δ is the diameter of a spherical volume equivalent to the volume of the initial suspension in 1 ml (μm); and d is the diameter of the particles (μm).

With a content of n_0 cells per 1 cm^3 in the initial suspension, the ratio $n_0/n = F$ shows the quantitative content of cells in the aerosol.

The particulate concentration of the aerosol in a chamber with uniform distribution throughout the entire volume will equal

$$C = \frac{n}{V} \text{ (particles/cm}^3\text{)}. \qquad (2.2)$$

The cellular concentration is calculated by the formula

$$C_0 = \frac{n_0}{V} = \frac{n \cdot k}{V} = K \cdot C \text{ (cells/cm}^3\text{)}.$$

The example given below demonstrates the sequence of preliminary evaluation of particulate and cellular concentration in a closed volume.

Let it be required to determine tentatively the particulate cellular concentration in a closed volume equal to 1 m^3 with dispersion of 1 ml of suspension containing $2 \cdot 10^9$ cells/cm^3. With dispersion of the sample that aerosol is created which is close to monodispersed and has a particle size of 10 μm. Representing the volume of initial suspension of 1 ml in the form of a sphere, we can determine the diameter of this sphere through the following expression:

$$d = \sqrt[3]{\frac{1 \cdot 6}{\pi}} = \sqrt[3]{\frac{6 \cdot 1}{3.1}} = 1.24 \text{ cm} = 1.24 \cdot 10^4 \text{ μm}.$$

Then the quantity of particles 10 μm in diameter which can be obtained during dispersion of 1 ml of suspension will equal

$$n = \frac{A \cdot V}{d^3} = \frac{1 \cdot (1.24 \cdot 10^4)^3}{10^3} = 1.9 \cdot 10^9 \text{ particles}.$$

Since the initial suspension contained $2 \cdot 10^9$ cells/cm^3, all of these cells are distributed in aerosol particles. The quantitative relationship between the number of cells and the number of aerosol particles will equal

$$K = \frac{n_0}{n} = \frac{2 \cdot 10^3}{1.9 \cdot 10^3} = 1{,}05.$$

If we hold to the assumption that the cells are distributed in the aerosol in such a way that there are no particles which do not contain cells and no particles containing more than two cells, then the value K = 1.05 shows that 5% of all particles contain two cells, while all the remaining particles contain one cell apiece.

Under the conditions of the example the particulate concentration will equal

$$C = \frac{n}{V} = \frac{1.9 \cdot 10^3}{10^3} = 1.9 \cdot 10^3 \text{ particles/cm}^3.$$

The cellular concentration will be

$$C_0 = K \cdot C = 1.9 \cdot 10^3 \cdot 1.05 = 2 \cdot 10^3 \text{ cells/cm}^3.$$

The above tentative calculations can be supplemented during consideration of the particular features of the experiment.

If no mixing of the aerosol occurs under the chamber conditions, the fraction of particles out of the total quantity settling in a determined span of time can be determined by the Stokes formula (1.2) under the assumption that there is virtually no settling of particles on the ceiling or walls of the chamber. Evaluation of the degree of coagulation of the particles and the change in particulate concentration due to this is carried out by the Smolukhovskiy formula (1.5), given in Chapter I.

The described method for evaluating cellular concentration is extremely approximate, since it does not take into account the viability of the cells.

The basic instruments used to determine the concentration and particle size of aerosols can be broken down into the following classes: instruments based on the principle of free deposition of aerosol particles; instruments based on the principle of the impact action of a jet of air; and instruments which ensure counting of aerosol particles in isolated volumes of air.

Since the principles upon which operation of these instruments are based are also used in apparatus for microbiological study of the air, it would seem advisable first to describe the common principles and methods, along with certain of the best-known instruments, intended for determining the concentration and degree of dispersion of aerosols.

The most important condition for determining aerosol concentration is correct selection of the method for taking samples of the aerosol. The majority of existing methods of sampling aerosols are based on the principle either of suction (aspiration) of particles into some sort of instrument, the principle of deposition of particles on various surfaces, or on a combination of these two principles - aspiration with subsequent deposition.

During aspiration of an aerosol essential changes arise both in its particulate concentration and in the nature of distribution of aerosol particle sizes as compared with the aerosol in the undisturbed state - i.e., prior to the moment of its aspiration into the instrument. These changes can occur in the collecting tube or in the instrument itself due to gravity deposition of aerosol particles out of the laminar and turoulent flow or because of diffusion of the aerosol. Therefore when taking samples of an aerosol for its investigation, it is important to know, however tentatively, the possible changes in its particulate concentration.

Some idea of the nature of the changes in particulate concentration and degree of dispersion of the aerosol during

sampling can be provided by consideration of the physical basis underlying the various methods of sampling.

a) Aspiration of Aerosols from an Undisturbed Volume

During sampling of an aerosol from undisturbed air there will be, as a rule, no losses due to the inertia of the aerosol particles. This is explained by the fact that the streamlines during such sampling are rectilinear. Virtually 100% effectiveness of sampling from unmoving air is obtained when the collecting tube is arranged horizontally or at a certain angle to the horizon. When a sample is drawn through a vertical tube open at the top the concentration of the aerosol in the sample will be greater than the true concentration by $[1 + (v_s/u)]$ times. This is explained by the fact that with this sampling procedure the velocity of the aerosol particles is the vector sum of the velocity of the medium (u) and the rate of deposition (v_s) (A. N. Fuks, 1955).

b) Taking Samples from a Flow

The basic factors which give rise to a change in the true concentration in an aerosol sample taken from a flow are the orientation of the collecting tube relative to the flow and the ratio of the flow velocity in the tube and that outside it. If the collecting tube will be arranged at a certain angle to the direction of the main flow, then because of inertia of motion a certain fraction of the particles will settle out on the inner wall of the tube. The concentration of the aerosol in the sample will be lower than the true concentration. When the collecting tube is arranged parallel to the flow the speed of flow in the tube may be greater or less than that in the main flow; in the first case the concentration will be overstated and, in the second, it will be understated. Taking of aerosol samples when

the speed of flow in the tube and that in the external flow are equal is called isokinetic sampling. With this method of sampling, if we ignore deposition of the aerosol on the face of the tube, the true concentration of the aerosol should be obtained.

However, studies have shown that even with isokinetic sampling an understated value is obtained for concentration of dust in the tube (Dennis, 1957). It was found that to obtain a more correct result it is necessary that the external pressure be greater than the internal pressure; this is achieved by increasing the rate of aspiration of the sample, with the increase being the greater, the higher the velocity of the external flow. The author explains this loss in pressure in the tube as due to friction and swirling.

A great number of studies have been dedicated to problems in taking samples of aerosols (May, 1945; Walton, 1954; Watson, 1954; Badrioch, 1959; Walter, 1957; Dennis, 1957).

The theory of sampling aerosols was developed very deeply by L. M. Levin (1961). In particular, he obtained formulas which can be used to calculate the effectiveness of drawing samples from a uniform flow of aerosol with consideration of inertia and of sedimentation of the particles. The formula for drawing a sample through a very narrow opening has the form

$$A = \frac{n}{n_0} = 1 - 0{,}8K + 0{,}08K^2 - \ldots \qquad (2.3)$$

$$K = \left(\frac{4\pi}{\Phi}\right)^{1/3} \cdot v_s (u_0 - v_s)^{2/3}.$$

where n_0 and n are the concentrations of the initial aerosol and of the sample; Φ is the volume of aerosol aspirated in 1 second; u_0 is the speed of the undisturbed flow; v_s is the rate of deposition of particles; and A is the aspiration coefficient.

Formula (2.3) is applicable under conditions when the average velocity of the flow in the collecting opening is substantially greater than four times the value of the speed of the undisturbed flow ($u > 4u_0$).

For sampling in an infinitely narrow flat slit, L. M. Levin obtained the following formula:

$$A = \frac{n}{n_0} = 1 - 0{,}451\,K - 0{,}148\,K^2 + \cdots \qquad (2.4)$$

$$K = \frac{2\pi\tau}{\Phi^1} \cdot (u_0 + v_s)^2,$$

where Φ^1 is the rate of sampling of the aerosol per unit length of the slit. In formulas (2.3) and (2.4) τ is the relaxation time of the particles:

$$\tau = \frac{\rho d^2}{18\eta}.$$

As is evident from formulas (2.3) and (2.4), evaluation of sampling effectiveness is conducted through the aspiration coefficient, which is equal to the ratio of the concentration of the aerosol in the sample to the initial concentration. However, determination of the exact value of a true concentration in an aerosol flow is extremely difficult. Therefore, despite the wide variety of theoretical and experimental works concerned with problems of sampling aerosols from a flow, this problem has not yet found its final resolution.

1. Instruments Based on the Principle of Free Settling of Aerosol Particles

The Stokes (1.1) and Stokes-Cunningham (1.3) formulas lie at the basis of the determination of particulate concentration and particle size by means of instruments based on the particle sedimentation principle.

Instruments based on spontaneous separation of aerosol particles from the dispersion medium include the Owens-II counter, the Green sedimentator (Green, 1934), and others.

The Owens counter is designed on the principle of free deposition of aerosol particles from a determined volume onto a horizontal surface. The aerosol particles are collected on cover glasses, where they are counted under a microscope and with a reticular ocular micrometer.

The number of aerosol particles settling out from 1 mℓ of air is determined by the formula

$$N = \frac{n}{a^2 \cdot h}. \qquad (2.5)$$

where N is the number of aerosol particles settling out of 1 mℓ of air; n is the average number of particles counted in one grid section; a is the linear dimension in centimeters to which one side of a grid square corresponds in the field of vision; h is the height of the vessel in which the aerosol particles settle (in centimeters).

The Owens counter makes it possible to determine particulate concentration in an aerosol at the moment when the sample is taken.

The Green sedimentator (Green, 1934) is an instrument designed along the lines of the Owens-II counter, but differing from it in simplicity of construction and manipulation (Fig. 1).

The instrument consists of a cylinder (a) with a height of 5 cm and diameter of 3.6 cm. The top of the cylinder is covered by lid (b). The cylinder is installed on a base (c) which contains one or two depressions for cover glasses (d). During sampling the cylinder, opened above and below, is raised and lowered

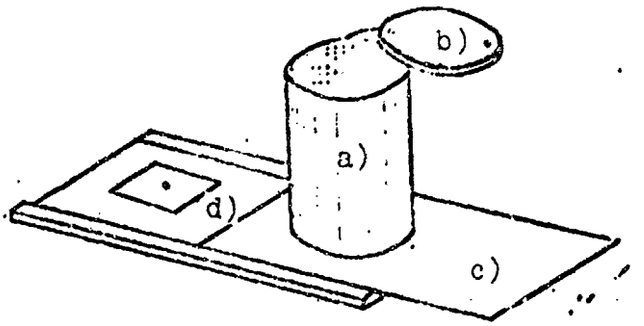

Fig. 1. Green sedimentator.
a) cylinder; b) cylinder cover; c) moving plate; d) base of instrument.

several times in air, after which it is set on the base under the next glass, covered with the lid, and left quiet. Calculation of the number of aerosol particles is carried out in the same manner as during use of the Owens-II instrument.

Among instruments based on the principle of free deposition of aerosol particles is the Pfeiffer aerosol trap (Pfeiffer, 1963). The instrument is intended for rapid sampling of an aerosol cloud. Aerosol particles enter a fixed volume - an automatically covered tank - and are trapped by corresponding surfaces through collisions with them and deposition on them. Plates or films intended for trapping aerosol particles upon collision are fastened to the inner surface of the vertical walls of the tank. A plate with a covering lid is included in the instrument to trap particles by deposition. During tests of the aerosol trap in field conditions it was noted that the instrument ensures obtaining the necessary research results independently of the state of the air flow and that it also makes it possible to take samples of aerosols within a flow in very short time intervals.

2. Instruments Based on the Principle of the Impact Action of an Air Jet

Calculation of aerosol particles under condition of mechanical separation of the dispersed phase from the dispersion medium can be carried out by means of instruments based on inertial deposition: konimeters of the Kotze design (cited in K. Spurny, 1964) and Owens-I (Owens, 1918, 1922) and with design changes - Miller and Sakhnovskiy (1931) and Bekhounek (1939), with a counter of the type designed by Torskiy, Volokhov, Kekin, and Radchenko (1951), the May cascade slot counter (May, 1945), the Stoyanovskiy dust counter (1951), with konimeters by Zeiss (Zeiss, 1956), Kleinschmidt (Kleinschmidt, 1935), and Lehmann, Love, and Franke (1934), with the aerosol trap by Hirst (1952) and that of Gregory (1954), etc.

The principle underlying the majority of these instruments consists in passing a strong jet of air through the instrument; this jet imparts kinetic energy to the particles and, impacting against adhesive plates, the particles stick to them. This energy is obtained as a result of an increase in the velocity of the air flow passing through a slit or a small round opening. Passing through the slit with a velocity of 10-100 m/s, the aerosol enters a rarified space where the speed and direction of the air jet are changed abruptly. Adiabatic expansion of the gas occurs, leading to condensation of water vapor on the particles and an increase in their weight. Due to inertia the particles strive to retain their initial direction of motion. Encountering an obstacle in the form of a glass plate, the particles are deposited on it. The rate of aspiration of air through the nozzle is increased to a magnitude at which the capture coefficient reaches 80-100%.

The effectiveness of inertial deposition (impactor) can be calculated by the formula (May, 1945)

$$imp = \frac{cpr^2}{\eta d_1}. \qquad (2.6)$$

where c is the speed of motion of the gas (in centimeters per second); ρ is the density of aerosol particles (in grams per cubic centimeter); r is the radius of a particle (in centimeters); η is the viscosity of the gas (in grams per cm per second); and d_1 is the width of the slit (in centimeters).

With constant density, particle size, and viscosity of the gas the effectiveness of inertial deposition is increased with a growth in the speed of motion of the gas within the gap and with a reduction in the width of the slit.

Instruments called konimeters have become widely known and used. A description of the principle of their operation is given below.

The Owens-I dustmeter. A certain volume of investigated air is aspirated into the instrument, where it is subject to adiabatic expansion and is cooled; moisture condenses on the particles which, impacting against glass which is placed in their path, adhere to the latter in the form of a thin strip.

Structurally the instrument consists of a housing, a moisturizing tube in the form of a cylinder, and a pump. On the upper end of the cylinder, whose inner walls are covered with wet filter paper (moistening tube), there is a cover with a slot 0.1 mm wide and 10 mm long. The tube is connected through this slot with a chamber, connected in turn with a piston pump which exhausts 50 cm^3 of air in a single piston stroke (Fig. 2).

The aerosol particles deposited on the glass can be counted by means of a microscope. To facilitate and accelerate counting of the quantity of particles, Kup (1942) proposed determining

tne number of particles contained in a single cross section of the strip and multiplying the obtained quantity by the microscope constant. The quantity of dust in the strip determined by this simplified method differs from the true quantity by no more than ±1.9%, according to Kup's data.

Eggloff (1933) established a relationship between the number of visible particles and the magnification of the microscope (Table 7).

Eggloff considers that with magnification of 1000 to 1500 times it is possible to see virtually all of the particles of an aerosol within the field of vision.

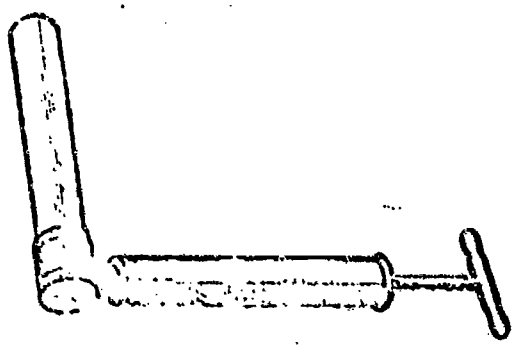

Fig. 2. Owens counter (external view).

Table 7. Relationship between microscope magnification and the number of particles visible in an Owens counter (per Eggloff, 1933).

Microscope magnification	100	250	500	1000	1500	2000
Counting results (per 1 cm)	65	102	113	112	124	125

Particulate concentration of aerosol particles in air is determined by dividing the volume of pumped air by the number of counted dust particles.

The total number of dust particles in 1 mℓ of tested air is determined by the formula

$$N = \frac{n \cdot L}{l \cdot v \cdot m}. \qquad (2.7)$$

where N is the average number of dust particles per 1 mℓ of tested air; n, average number of dust particles in one transverse strip; L is the length of the entire dust tract (in centimeters); l is the size of the side of one square in the field of vision with a given optical system (in centimeters); v is the volume of air pumped during one piston cycle (in cubic centimeters); and m is the number of piston cycles.

It is assumed that the mean error obtained by the Owens method amounts to $\pm 4\%$.

Kotze konimeter. The operating principle of the instrument consists in pumping air through a conical slot with a round cross section; the slot diameter in the lower portion equals 0.072 cm, and the speed of the air jet is 1150 cm/s. Aerosol particles undergo inertial deposition on a moving glass substrate. 8-12 samples are taken on one and the same substrate. The substrate is covered with a thin layer of glycerin or some other sticky material. The Kotze konimeter is at present being replaced by improved specimens in the form of Zeiss konimeters (English) and Bausch-Lomb instruments.

Zeiss konimeter consists of the following basic parts: sampler with an inlet channel 0.5 mm in diameter, along which 0.1 mm dust particles travel; rotating round object glass with an area calculated for taking 30 samples; an air pump exhausting 125 cm^3 of air in one piston stroke. The air is aspirated at a rate of 100 m/s and falls on the object glass, which is smeared with collodion or with glycerin mixed with rubber.

According to data from Glawion (1938) and Junge (1951), up to 7-8 times more particles are retained on a layer of gelatin than on a layer made of a mixture of glycerin with rubber.

On the slide the particles formed a coating in the form of a spot with an area of about 0.3 mm^2. The number of dust particles is determined visually in the illuminated field of the microscope or by the photometric method. Counting can be carried out at a substantial interval of time after taking of the sample. The most favorable conditions for counting are created with a dust-particle content in the sample of $5 \cdot 10^3$ (Reeger, Siedentopf, 1950), or of $15 \cdot 10^3$-$40 \cdot 10^3$ (Effenberger, 1940). Therefore the recommended volume of the air sample, containing highly concentrated aerosol, should amount to 1.25 cm^3, while for air with a small quantity of particles several liters should be taken.

The relative accuracy of konimetric measurements falls in the limits $\pm$7-14% (Effenberger, Reeger, etc.).

Determination of dust content by calculating the number of particles in a dust spot on a measuring glass is accomplished by various methods. It has been established that a spot formed as the result of deposition of aerosol particles contains a number of particles which gradually diminishes from the center to the periphery. The following methods are used to carry out the counting: the Rotshke method of annular counting (1937), the Lobner method (1935) and the Effenberger photometric method (1940).

Thus, Effenberger (1952) described a konimeter with a recording device for making a continuous recording of measurement results over a comparatively prolonged period.

The Ma impactor (1945) is a substantially superior instrument to the Owens counter; this instrument is a highly

effective aspirating collector. By using this instrument it is possible to determine both the particulate concentration of the aerosol and also its degree of dispersion. This instrument contains not one trapping stage, but four of them (Fig. 3).

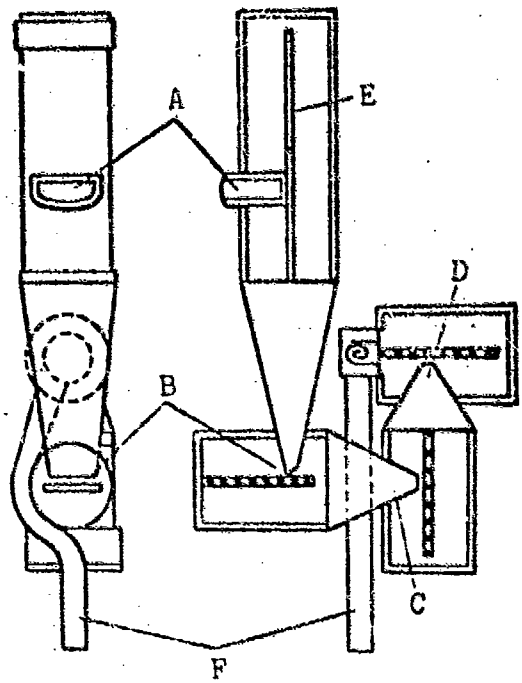

Fig. 3. Diagram of a four-stage May impactor (see description in text).

As is evident from Fig. 3, the May cascade impactor consists of a system of four nozzles (A, B, C, D) and trapping plates corresponding to them. The diameter of the inlet nozzles is progressively reduced from stage to stage so that the speed and consequently the effectiveness of particle trapping is increased from plate to plate. As a result the particles are sorted by dimension, which greatly eases subsequent analysis of the samples. The May instrument is portable, reliable in operation, and can be used to take samples of any aerosols whose particle sizes exceed 1 μm. The optimum rate of air aspiration is 17.5 *l*/min. Before air samples are taken the plates are coated with a sticky

film of non-drying substances, in particular a mixture of castor oil with highly refined resin. Analysis of the samples is carried out by means of a microscope with an ocular micrometer. However, tentative determination of the degree of dispersion of the particles in the samples is possible without the use of the ocular micrometer.

Thus, it has been noted that during passage of an aerosol through four wedge-shaped nozzles with slot widths of 6, 1.6, 1.0, and 0.6 mm, the air flow is separated from them at velocities equal to 5, 30, 50 and 80 m/s, respectively. Since the effectiveness of deposition of particles suspended in a gas flow grows with an increase in flow velocity and in size of the particles, aerosol fractions of sizes of 10-200, 3-20, 1-7, and 0.7-3 μm can be obtained on glass screens placed behind each of the slots.

In the isokinetic mode the May impactor can serve as a standard for calibrating other samplers of similar type.

In 1956 May designed an improved model of the cascade impactor, equipped with moving plates. This instrument makes it possible to study the concentration of aerosol particles in time, since the deposit formed on the plates is not excessively dense and does not hamper microscopic study of the aerosol particles.

Wilcox (1953) proposed a five-stage cascade impactor.

The Hirst trap (1952) consists of a single impactor stage (Fig. 4).

The instrument is intended for deposition of aerosol particles with consideration of the air being passed through. Particles are deposited on a slide which moves at a speed of 2 mm/h (3) by means of clockwork mechanism (1). In 24 hours

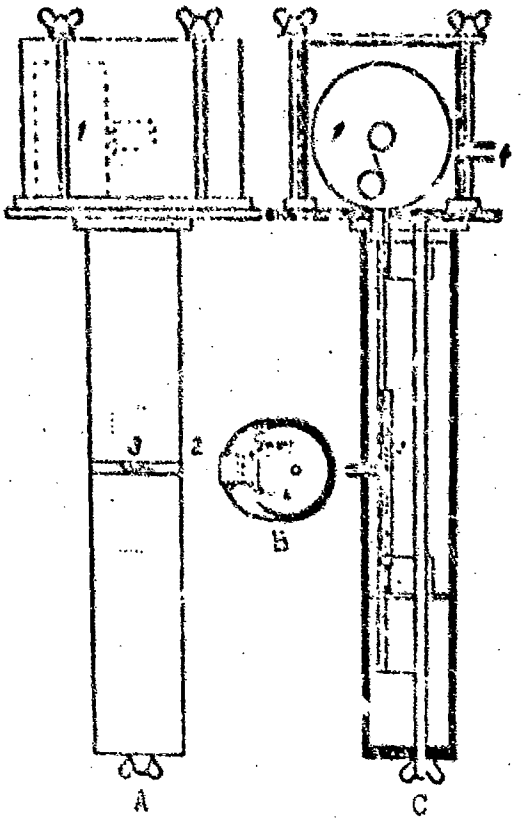

Fig. 4. Hirst trap (diagram). A - front view (upwind side); B - cross section to the intake slot; C - side view (longitudinal section). 1 - clockwork mechanism which moves the plate; 2 - intake slot, turn to face the wind, behind which the holder containing plate (3) moves upward; 4 - to the vacuum pump. (Weathervane unit and cap for protection of the inlet opening from rain are not shown).

the particles are deposited on the glass in the form of a strip 48 mm long. The slide is arranged behind the slot (2). Microscopic examination of the deposit on the glass makes it possible to obtain some idea of both the mean daily quantity of particles and also of the quantity of particles settling out of the air in given intervals of time (± h). Sampling can be accomplished isokinetically in the Hirst trap. Indisputable advantages of this instrument includes simplicity of design, ruggedness, and the possibility of a continuous operating mode. However, the instrument does not allow taking samples on a nutrient medium.

The Gregory instrument (Gregory, 1954), like the automatic Hirst trap, is intended for deposition of aerosol particles (spores) with consideration of the volume of air aspirated through the instrument. The aspiration rate is 10 l/min of air. The instrument does not require electric power for its operation, and aspiration of the air is accomplished by manual rotation of a small pump with sliding vanes. The apparatus weighs 4.5-5.5 kg. Advantages of the instrument include the following: portability and the possibility of taking air samples with a duration of up to a second. The Gregory trap is not intended for samples on nutrient media.

The action of the aerosol counter designed by A. F. Stoyanovskiy (1951) is based on the use of centrifugal force, causing the particles to collect on the inner surface of a rotating drum. The instrument consists of a hollow cylinder which can be opened into two halves. The cylinder is mounted on the axle of a fractional-horsepower motor, to which a handle is fastened. On the inner side of each valve there are cover glasses which are smeared with a layer of castor oil. At the point where the air is to be investigated the cylinder is open for several minutes to permit achievement of equilibrium between the aerosol concentration in the cylinder cavity and that in the external atmosphere. Then the cylinder is closed, the covering glasses are opened, and the electric motor is switched on. The cylinder is rotated at a speed of 2000 r/min; this carries the particles of the aerosol close to the surface of the covering glasses, where they are fixed. Deposition of the particles is accomplished within 3-5 minutes. Counting of the number of aerosol particles is carried out with a microscope. The quantity of aerosol particles per unit volume is determined according to the formula

$$N = \frac{n}{1.25 \cdot 2^2 \cdot m}. \qquad (2.8)$$

where N is the quantity of particles per ml of air; n is the average quantity of particles deposited on the area of the ocular grid; a is the side of the ocular grid at a given magnification, expressed in centimeters; m is the quantity of air samples taken from which aerosol is deposited on one or another glass; 2.75 is the volume of air (in milliliters), from which aerosol is deposited on an area of 1 m^2.

Among the basic drawbacks of the instrument we must include disturbance of the physical structure of the aerosol in the process of sampling.

Traps in which aspiration of the air is carried out by means of a pump, fan, or aspirator will operate, to a certain degree, independently of wind speed and particle dimension and can consequently be utilized in open-air conditions.

A number of essential drawbacks are inherent to instruments of the Owens type and to certain other inertial samplers: aggregation of particles occurs on the preparations, while during passage of the aerosol through the slot breakdown of conglomerates into individual particles is possible (Ye. Vigdorchik and M. Pavlova, 1933). Application of counters is also impossible when large aerosol particles are present (larger than 100 μm), since the slot of the chamber becomes plugged; they are not suitable for operation with aerosol particles which wet poorly and they provide inadequate trapping of fine particles (A. I. Pakhomychev, 1939; M. N. Krasnogorskaya et al., 1939).

In addition, when inertial traps with forced aspiration of the air are used, difficulties arise in trapping all particles of an aerosol; these difficulties are connected with the isokinetic conditions of sampling. Not all of the particles which enter the slit turn up on the surfaces intended for them; some of them

are deposited on the walls of the trap and another portion is not trapped at all and passes on through the trap.

Konimetric methods also have drawbacks. Therefore Junge (1952) recommends the introduction of corrective factors to eliminate the effect of aspiration rate and other factors when utilizing the konimetric method. Fairly good results have been obtained by certain investigators with the use of the electro-precipitation method.

The ability to carry an electrical charge is an important property of aerosol particles. This property provides the basis for the method of precipitation of charged particles under the influence of an electrical field.

An aerosol particle can be charged during triboelectric phenomena (electrification by friction) or by capture of gas ions. These two forms of charging of aerosol particles are used in various instruments whose operating principles are based on electrical deposition of charged particles in an electrical field. Instruments in which charging of the particles occurs due to the friction over a dielectric include, in particular, the triboelectric konimeter described by P. N. Porskiy in 1953. The instrument is used for determination of aerosol concentration.

Charging of aerosol particles by capture of gas ions is applied mainly for deposition of particles in electrostatic precipitators, in particular an electroprecipitators and electrical filters. Usually the source of ions in these instruments in spontaneous (corona) discharge. A positive corona is used in industrial electrical filters, with the aerosol particles obtaining a positive electrical charge in them. A negative corona is used in electroprecipitators, and during capture of ions the aerosol particles are charged negatively.

The negative electrode (cathode) in the instruments is usually made in the form of a set of thin wires or needles, while the positive electrode (anode) is in the form of a flat or cylindrical surface, which is usually the substrate.

The potential difference between electrodes at which spontaneous discharge sets in amounts to several tens of thousandths of volts. Passing through the region with a high density of negative ions, the aerosol particles are charged by capture of negative ions and are then deposited on the anode.

The electroprecipitator consists of three basic parts: a high-voltage source, a chamber for precipitation of particles, and a pump or blower.

Among Soviet electroprecipitators, widely known instruments are those designed by Blinov and Litvinov (1951), and that by Trukhanov (cited in A. I. Burshteyn, 1955).

Luckiesh, Taylor and Holladay (1946) designed an electrostatic trap. Later different types of electrostatic traps were proposed by Rack (1959), O'Connel, Wiggin, Pike (1960) and others.

Counting of particles in electroprecipitators can be carried out by the formula

$$N = \frac{n \cdot S}{a^2 \cdot h}, \qquad (2.9)$$

where N is the number of particles per ml of air; n is the average number of particles in one grid square (particles/cm^2); S is the area on which particles are precipitated (in square centimeters); a is a linear dimension representing the side of a grid square in the field of vision (in square centimeters); and h is the height of the chamber (in centimeters).

The possibility of utilizing the principle of electroprecipitation for bacteriological research on the air is described in Chapter III.

Thermal precipitation was proposed comparatively recently as a means of determining concentrations of aerosol particles. The principle underlying thermal precipitation is as follows: If a heated body is placed in a chamber containing aerosol particles, a zone will be formed around it which is free of the aerosol. This phenomenon is due to radiometric forces acting on nonuniformly heated bodies located in the medium; methods for calculating these forces are outlined in detail in works by N. A. Fuks. The physical basis of this phenomenon is used in trapping aerosol particles by thermal precipitators. The operating principle of the thermal precipitators consists in trapping of aerosol particles on substrates located between hot and cold surfaces. The difference between the temperatures of the hot and cold surfaces amounts to about 100°.

The method of thermal precipitation has a number of advantages over inertial deposition. The advantage lies in the fact that the rate of motion of particles 0.1 μm and larger in size within a thermal precipitator depends little on their size, while the rate of motion under the action of gravity and inertia diminishes rapidly with a reduction in particle size. Besides this, during thermal precipitation there is no crushing of aggregates and blowing about of deposited particles.

Spurny et al. (1964) noted that in thermal precipitators all particles less than 20 μm in size are captured.

The effectiveness of the trapping of aerosol particles in thermal precipitators depends on the rate at which air is drawn through the instrument, the temperature gradient between the surfaces in the thermal precipitator, and the thermal conductivity

of the medium. Besides this, Green and Lane (1957) noted that this method gives best results at a high particle concentration (volume of air drawn through the unit comprises only 7 cm/min [sic]).

The first Soviet thermal precipitator was designed by P. N. Torskiy (1951). The instrument consists of three basic parts: the precipitator housing, the aspirator, and a current source with ammeter and rheostat for regulation of current strength. Passing 100-200 ml of air through the instrument, Torskiy obtained 100% trapping of solid aerosol particles.

Application of the principle of thermal precipitation for purposes of bacteriological study of the air is described in detail in Chapter III.

3. Counting Aerosol Particles in Isolated Volumes of Air

Determination of aerosol concentration by the methods described above (for example, deposition on plates) with subsequent counting of the particles under the microscope is complex and takes a great deal of time.

Of significant interest are the efforts of certain authors to apply physical methods of investigation (Ye. A. Vigdorchik, 1933; B. V. Deryagin and G. Ya. Vlasenko, 1951; V. S. Kiktenko, Yu. P. Safronov, S. I. Kudryavtsev et al., 1961; A. I. Danilov, Yu. P. Pokhitonov, 1966; Guyton, 1946; Gucker, O'Konski, 1949; Ferry, Farr and Hartmann, 1949; Klánovice, 1957, 1960).

The following particular features are characteristic of these methods of counting aerosol particles: the dispersed phase of the aerosol is not separated from the dispersion medium, in order to avoid aggregation of the particles; counting of particles is carried out by means of a slit ultramicroscope, where the aerosol

particles are represented in the dark field in the form of glowing points; counting of the particles is carried out only in an optically separated volume of the vessel; the average number of aerosol particles per unit volume is determined by appropriate calculations based on multiple counting of particles in the optically separated volume.

For rapid determination of the quantity and magnitude of aerosol particles in instruments designed on these principles use is made of converters (optical, electrical, etc.), which develop an electrical impulse of different intensity for each particle. The magnitude of these pulses is found to be directly dependent on particle size (in particular, on the magnitude of the surface, mass, etc.). The descriptions of the most widely known and applied Soviet and foreign aerosol particle counters are presented below.

The counter designed by Ye. A. Vigdorchik consists of the following basic parts: microscope, illuminator, and cuvette. Besides these basic parts, there are a number of attachments to facilitate focusing of the light in the cuvette, attaching the individual parts, etc. The instrument makes it possible to use the speed of fall of particles to determine their size. Consequently the instrument determines not only the particulate concentration of the aerosol, but also its degree of dispersion. The counter is used to determine the number of particles in highly dispersed aerosols under production conditions and has demonstrated its suitability for these purposes.

In 1946 Guyton developed an electrostatic counter. The operating principle of the instrument consists in the appearance of electrical impulses as a result of impact on a copper collector filament 0.4 mm in diameter of aerosol particles traveling at high velocity in a thin stream. The electrical vibration imparted to the collector by the particles (particle diameters 2.5 μm

and more) is amplified by an hf amplifier by 100,000 times and activates a mechanical counter. Guyton found that the amplitude of vibrations is proportional to the diameter of the aerosol particles. Determination of particle size is based on the essential dependence of the magnitude of voltage on the square of the particle diameter. The Guyton instrument can count up to 1800 particles per minute. The instrument records particles with dimensions of 2-3 μm; however, with a change in the type of amplifier it becomes possible to increase the sensitivity of the instrument and to determine particles 0.3-1 μm in size.

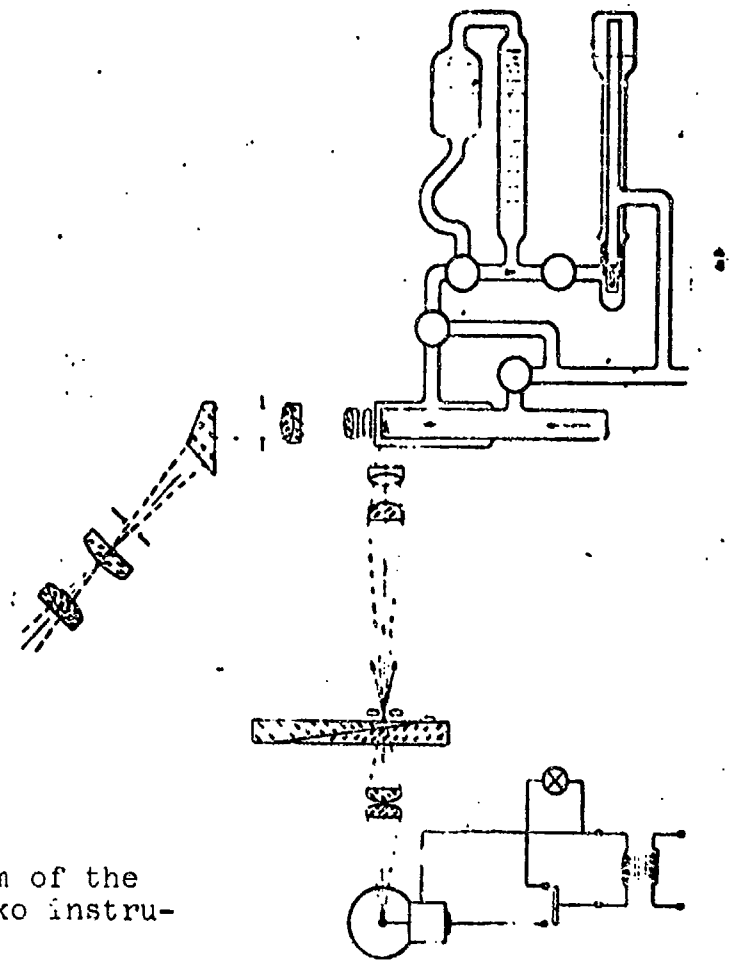

Fig. 5. Diagram of the Deryagin-Vlasenko instrument.

B. V. Deryagin and G. Ya. Vlasenko (1948) developed a flow method for counting aerosol particles. Figure 5 shows a diagram of the device. The principle underlying the B. V. Deryagin and G. Ya. Vlasenko method of flow ultramicroscopy consists in counting particles in a continuous flow of aerosol. The particles cross the illuminated zone in a certain span of time. Counting is carried out according to "flashes" arising at the moment when individual particles intersect the illuminated zone. Particulate concentration of the aerosol is calculated on the basis of the total number of particles counted and the volume of aerosol passing through the illuminated zone. Determination of the fractional composition of the dispersed system is achieved by counting particles at a constant rate of flow but with different and gradually diminishing illumination. Reduction of illumination in the counting zone is achieved by introducing a wedge on the path of the illuminating beams. With a reduction in illumination the eye is capable of registering only particles with a radius larger than a determined size. By progressively reducing the illumination in the counting zone it is possible to successively eliminate from the count those particles which scatter light with inadequate intensity. The size of these particles grows as the light illuminating the counting field is damped. Thus it is possible to study the fractional composition of the dispersed phase of the investigated aerosol. It should be noted that in this method of determining the fractional composition of the dispersed phase errors can arise in connection with the fact that the force of the light source and the sensitivity of the eye are not constant.

The possibility of relatively rapid visual observation of particles (of a bacterial aerosol) by means of the flow ultramicroscope was put forward by V. S. Kiktenko and coworkers. They found indisputable advantages in using the flow ultramicroscope as compared with earlier methods of investigation.

In order to replace the eye of the observer with a more sensitive photoelement, in order to allow automation of the entire process of particle counting, V. S. Kiktenko, Yu. P. Safronov, S. I. Kudryavtsev et al. (1961) proposed using a high-sensitivity photoelectron installation which, together with the electromechanical counter, makes it possible to count the quantity and determine the sizes of aerosol particles passing through the cuvette of the flow ultramicroscope. The magnitude of the light flux scattered by the particles is adequate for its recording by means of contemporary industrial photoelectron multipliers of the types FEU-19, FEU-25, etc. As observations have shown, the duration of a light impulse from the particle does not exceed 0.5-0.6 seconds, while the pulse arrival frequency depends on the quantity of particles and does not exceed 300-400 pulse/min.

The principle of recording pulses of a flux scattered by particles was used to design and test under laboratory conditions and experimental installation which confirmed the possibility of automating counting of aerosol particles. The installation consists of the following units: a photoelectron attachment to the ultramicroscope, a pulse counter, an amplifier, and a power source.

The electronic circuit of the installation is shown on Fig. 6.

The photoelectron attachment is intended to convert the light flux from the aerosol particles into an electrical impulse and to provide preliminary amplification of the electrical signal. It consists of a photomultiplier and a preamplifier. Structurally the attachment is made in the form of a separate unit whose input window is placed tightly against the ocular of the ultramicroscope (Fig. 7).

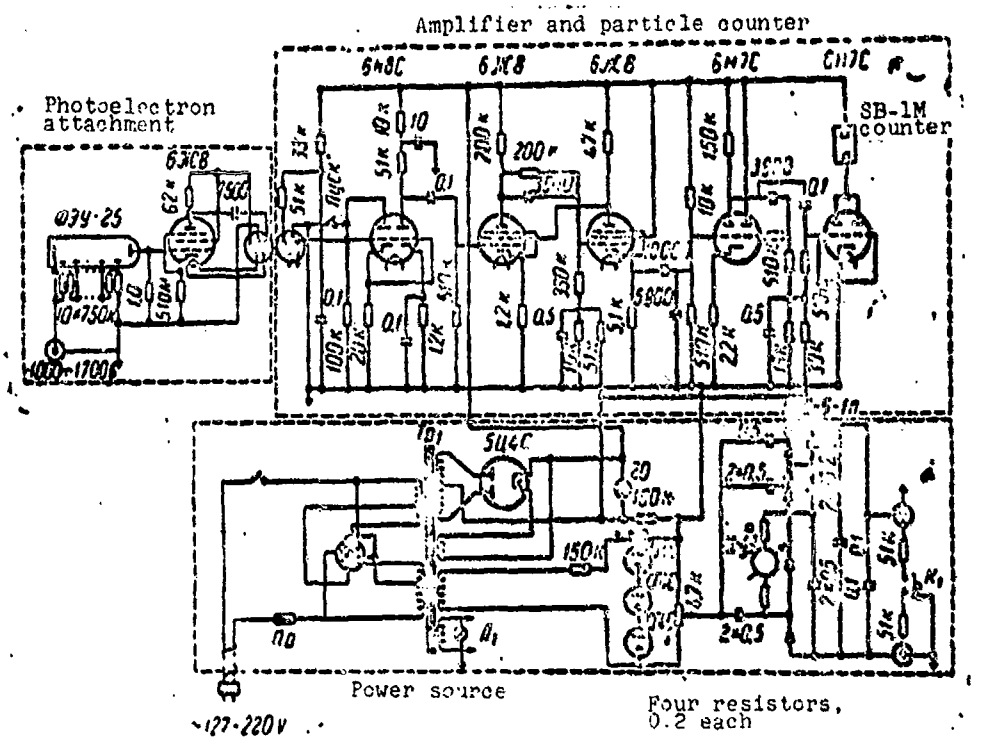

Fig. 6. Circuit of the photoelectron attachment (see description in text).

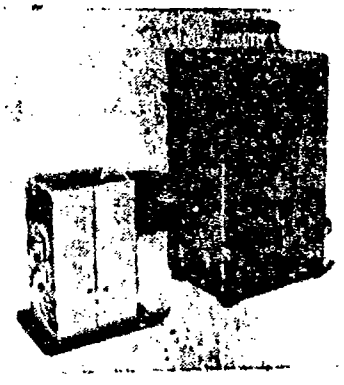

Fig. 7. Working position for the photoelectron attachment and the ultramicroscope.

The amplifier and the pulse counter are intended for amplification of electrical pulses to a level adequate to trigger an electromechanical counter of the SB-1M type. The amplification and pulse-counter unit consists of an input gate stage and an amplifier-limitor, a blocked input multivibrator, an output multivibrator, and an amplifier stage which activates the electromechanical counter. The high voltage for supplying the photomultiplier, anode voltage, and the voltage required to ensure filament heating are developed by the power source.

The amplifier, pulse counter, and power source are joined structurally into a single unit which is connected by cables to the photoelectron attachment.

When a light flux scattered by aerosol particles arrives at the cathode of the photomultiplier, an electrical pulse arises on its output and is amplified in the preamplifier and fed to the input gate stage of the amplifier. After amplification to the required magnitude the pulse passes to the electromechanical counter and triggers it. The pulse repetition rate at which the counter carries out error-free counting comprises approximately 100 pulse/s (6000 pulse/min), substantially exceeding the maximum possible particle frequency in the ultramicroscope. This ensures reliable recording of the number of particles. An error in the count due to inertia of the circuit can arise only when two or more particles appear in the field of view of the instrument within a time interval less than 0.01 seconds. For practical purposes such events are of low probability and therefore the error due to this factor does not exceed 1%. To ensure high measurement accuracy and to increase the sensitivity of the instrument it is necessary to turn attention to increasing the contrast of the particles by striving to ensure a low level of light flux from the background of the instrument viewfield.

Figure 8 shows an installation for automatic counting of aerosol particles, consisting of an ultramicroscope and a photoelectron attachment to which an amplification and particle-counting unit is attached. The setup is supplied with power from a net with a voltage of 127 or 220 V.

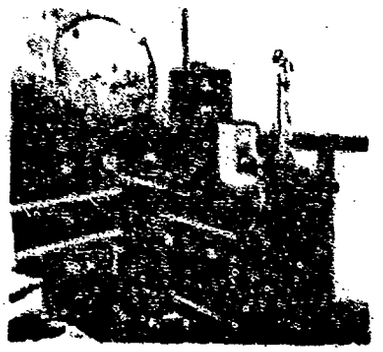

Fig. 8. Installation for automatic counting of aerosol particles.

If necessary an industrial installation of the B-2 type can be used as the amplifier and pulse counter, as well as the power source, with minor changes in the circuit; the latter are clear from the electronic diagram of the instrument given on Fig. 6.

A photoelectron counter was also developed by Gucker and O'Konski and subsequently substantially improved by O'Konski. In this instrument scattered light from each particle 0.6 μm and more in diameter strikes a photocell, giving rise to an electrical pulse which, after amplification by 200,000 times, triggers a mechanical particle counter. In this instrument aerosol particles are counted at a rate of 1200 counts per minute. The introduction of a differential pulse collector within the limits of the established voltage makes it possible to determine the degree of dispersion of the investigated aerosol.

Among other presently existing instruments intended for counting particles in determining their size in aerosols, we should mention the instrument developed by Alexander and J. Klánovice.

In 1955 Alexander designed an instrument for counting and measuring aerosol particles of various types (dust, radioactive, bacterial, etc.). The instrument, called an aerosoloscope, counts up to 100 aerosol particles per second. The aerosoloscope counts and measures aerosol particles in a size range of 1 to 64 μm up to 1000 times faster than can possibly be done with the present widely used methods of collecting particles on surfaces and studying them under the microscope. The operating principle of the instrument is also based on recording of light scattered during passage of a light beam through an aerosol system. The investigated aerosol system, with particle concentration up to 15,000 particles per cm^3, is diluted in the

instrument to such a degree that the instrument records every particle. Particle size is determined as a function of the quantity of light reflected by the particle. Electrical impulses pass from the photomultiplier to a recording system with two dials, where they are recorded successively in growing order from 1 to 64 μm.

This device is complex, since each quantity - i.e., each required group of particles - needs a separate sensing, amplifying and computing channel. Instruments of this type cannot be made portable, since they are very large in size.

Klánovice (1960) proposed an instrument with a unified sensing, amplifying, and recording channel. The instrument makes it possible to separate aerosol particles into any number of groups with continuous recording of the readings. Electrical pulses obtained in the converter of the instrument have an amplitude which is proportional to the size of the particles; these pulses are registered by an acoustic sensing device and then by a dividing attachment whose sensitivity is established in accordance with the magnitude of the amplitude of the received pulses. Pulses with identical amplitudes are successively selected and counted together. Recording of pulses is carried out on magnetic tape, where a signal track with different intensity is formed. This instrument permits rapid determination of the dispersed composition of the investigated aerosol and also of its concentration per unit volume of air.

In comparison with different microbiological quantitative methods for determining the concentration of bacterial aerosol particles, the application of photoelectron counting devices does have drawbacks, along with its advantages. Thus counting the quantity of aerosol particles occurs without a change in their aggregate state - i.e., bacterial agglomerates are not broken up in the air flow and the integrity of their structure

is not disturbed - a situation which arises, for example, when liquid filters are used; particulate concentration of the aerosol is determined almost instataneously and for a virtually unlimited time of study; the counters make it possible to record the most significant concentrations of particles of a bacterial aerosol, right down to single bacterial cells. At the same time, when photoelectron counters are used to determine the concentration of a bacterial aerosol it is necessary to consider that the majority of existing instruments count all of the particles in the air, without exception - they count particles of both organic and inorganic origin. The separation from the total flow of the aerosol of only those particles which contain bacterial or viral cells and, to an even greater extent, determination of the degree of their viability, presents substantial difficulties. In laboratory conditions these obstacles can be overcome to a certain degree by creating in the chamber an aerosol in which the concentration of initial suspension would ensure that every aerosol particle would contain one or several microbe cells. In this case the application of the particle counter permits continuous determination of the concentration of the bacterial aerosol and also of its degree of dispersion.

Above we have given a brief description of the most widely known and used methods and instruments based on them for determining the particulate concentration and degree of dispersion of various aerosol systems. One of the most essential drawbacks of the majority of these methods is disturbance of the structure of the investigated aerosol at the moment when it enters one or another instrument. As the aerosol is drawn into the instrument there is disturbance of the dispersed phase and of the dispersion medium, which leads to changes in their quantitative relationship. As a result the particulate concentration determined by means of the given instrument will not correspond to the particulate concentration of the aerosol in the medium from which the sample is drawn.

Determination of the greatest number of particles per unit volume of aerosol by means of one or another instrument also cannot be characterized as having a high degree of confidence. We must consider to be the most valuable method that one which gives figures approximating the real concentration of the aerosol as characteristic for the given dispersed system. From this point of view we should consider the best counting methods to be those with which sampling is carried out not by aspiration of aerosol into the instrument, but rather by isolation of a predetermined volume of air.

CHAPTER III

INSTRUMENTS FOR SANITATION AND BACTERIOLOGICAL INVESTIGATION OF AIR

The determination of particulate concentration of biological aerosols is based on the same principles and encounters the same difficulties as the measurement of concentration of inorganic aerosols.

In air microorganisms can fulfill the role of condensation nuclei; by coagulation they can form aggregates with both organic and inorganic particles of dust and by joining together.

For more than 100 years a huge volume of studies have been carried out on the subject of developing methods for studying bacterial seeding of the air. The methods of making air samples are of critical importance in determining the laws governing its microbe contamination, means of transmission of the causative agents of respiratory diseases, etc. Numerous bacterial traps of widely varied design have been proposed for bacteriological study of the air. However, at present there is no single procedure for trapping microorganisms from the air which is widely accepted in hygiene and bacteriological practice, despite the fact that application of different methods of research will, in the majority of cases, give results of poor comparability.

Table 10. Classification of bacterial traps (V. S. Kiktenko, S. I. Kudryavtsev).

Principles forming the basis of instrument operation	Name of the media used to trap microorganisms	Varieties of instruments and methods of sampling	Authors, year
1	2	3	4
DEPOSITION OF DISPERSED PHASE:			
By gravity	Neutral sticky substances	Glass plates	Salisbury, 1866
(Isolated volume of air)	Nutrient medium: meat bouillon,	Glass bottles	Pasteur, 1860
The same	fish glue	Glass plates	Fodor, 1881
" "	Agar-agar and other solid nutrient media	Glass Petri dishes	Koch, 1881
			K. P. Koval'kovskiy, 1885
" "	" "	Bacterio-logical trap	Alvarer and Castro, 1952; S. I. Kudryavtsev and coworkers, 1966

Table 10 (continued)

Impact of air jet (Nonisolated volume of air)	Sticky neutral substances (vaseline, gelatin-glycerin, mixture of vaseline with paraffin, silicone, etc.)	Glass plates (instruments of the aeroscope and aerokoniscope types)	Salisbury, 1866; Maddox, 1870; Cunningham, 1873; Airy, 1874; Miquel, 1878 Scheppegrell, 1922; Christoff, 1934
The same	The same	The same	Shitikova-Rusanova (per Stepnov, 1935)
" "	" "	" "	Hyde and Williams, 1943
" "	" "	" "	Durham, 1944
" "	" "	" "	Davies, 1951
" "	" "	" "	Green and Lane, 1957
" "	" "	Cascade impactor	May, 1945
" "	" "	Automatic trap	Hirst, 1952
" "	" "	Portable trap	Gregory, 1961
Impact action of air jet	Solid nutrient medium	In tubes of various construction	Hesse, 1884 A. Pavlovskiy, 1885
(Nonisolated volume of air)	The same	The same	N. Keldysh, 1886; Frankland, 1887

Table 10 (continued)

The same	The same	In Petri dishes (settling surface)	P. N. Matveyev, 1951, V. S. Kikhtenko and coworkers, 1960
" "	" "	In Petri dishes	Gregory, Stedman, 1953
" "	" "	The same	Richards, 1955
" "	" "	" "	Werff, 1958
" "	" "	Sampling with automatic machines	A. F. Stoyanovskiy, V. V. Reva, 1954
The same	The same	Sampling under field conditions	A. I. Vasil'yev, 1957
" "	" "	Sampling of high-altitude air	Ya. G. Kishko, 1959
" "	" "	Sampling in field conditions	V. M. Khil'ko, 1959
Inertial deposition:	Solid nutrient medium	Funnel-shaped instrument	Hollaender Dalla Valle, 1934
(Centrifugal action)	The same	Screen-type instrument	Du Buy, Crisp, 1944
The same	" "	Aerocentrifuge	Wells, 1933; A. I. Shafir, 1945

Table 10 (continued)

The same	The same	Aerocentrifuge	S. S. Rechmenskiy, 1951
(Slit method)	The same	Slit sampler	Bourdillon, Lidwell, Thomas, 1942
The same	" "	The same	Yu. A. Krotov, 1951
" "	" "	" "	Schuster, 1948
" "	" "	" "	Kuehne, Decker, 1957
" "	" "	" "	Lidwell, 1950
" "	" "	" "	Zampach, 1959
" "	" "	The "monitor" cascade cup impactor	Andersen, 1958
" "	" "		A. Andersen and M. Andersen, 1962
Combined slit and liquid methods	Solid and liquid nutrient media		Lazowski, Kancelarczik, 1956
The same	Solid and liquid nutrient media, gelatin filters, membrane filters	General-purpose instrument for sampling microflora, dust, and gaseous impurities in the atmosphere	Ya. G. Kishko, 1959

Table 10 (continued)

Electrostatic forces	Solid nutrient medium	Electroprecipitators	V. I. Vartanov, 1888
The same	The same	The same	Berry, 1941
" "	" "	" "	Rooks, 1948
" "	" "	" "	N. D. Uspenskiy and K. Lebedev, 1948
" "	" "	" "	Houwink, Rolwink, 1957
" "	" "	" "	O'Connell, Wiggin,
" "	" "	" "	Pike, 1960
Combination of electrostatic precipitation, impact action of air jet, and filtration through liquid	Solid and liquid nutrient media	Hydroaeroscope	Maisonnet, 1956
Combination of electroprecipitation and impaction	Solid medium	Electrostatic aeroscope	Symon and Bynek, 1964

Table 10 (continued)

Thermal precipitation	Cover glass; filter paper or strip impregnated with agar	Thermal precipitators	Kethley, Gordon, Orr, 1952 Orr and Martin, 1958
Solid insoluble filters	Cotton filters		Pasteur, 1860
The same	Filter paper		Rubner, 1907
" "	The same		Huberndick, 1906
" "	Paper filters		Sargent, 1907 Gonnel and Thoma, 1925
" "	The same		Mironov, Krauze, Boiko, 1940
" "	Wadding filters (cotton and glass wool)	Glass tubes with cotton wool	Pasteur, 1861 Rousbery, 1947
" "	The same	Metallic tube with cotton wool; "pistol instrument"	V. A. Zubarev, 1954 Ya. G. Kishko, V. I. Filimonov, 1958
" "	" "		
Solid insoluble filters	Cotton-wool filters with washable impregnants (mixture of 3% gelatin and vaseline oil)	Glass adapter	V. S. Kiktenko, N. I. Kashanova, S. I. Kudryavtsen, N. I. Pushchin, 1961

Table 10 (continued)

The same	Membrane filters of nitrocellulose	Instrument of the Seitz filter type	P. F. Milyavskaya, 1945; Ya. B. Reznik, 1951
" "	The same	The same	Albrecht, 1957
" "	" "	" "	Spurný, 1964
" "	Membrane filters of the "multipore" type (ash-free cellulose)	" "	First, Silverman. 1953
" "	The same	" "	Goetz, 1953
The same	"Mikrofil"-type filters (FPP-5-8 filter)		B. F. Sadovskiy, V. V. Vlodavets, Ye. Yu. Zuykova, L. I. Mats, N. V. Petryanov, 1963
Solid soluble filters	Sea salt Sodium sulfate, sugar, sodium acid phosphate, Magnesium sulfate		Foll, 1885 Miquel, 1883 F. F. Lapchinskiy, 1896
The same	Gelatin filter		Mitchel, Timmons, Dorris, 1959 A. Ye. Vershigora, 1957 V. M. Shul'zhenko, A. A. Antonove, 1959

Table 10 (continued)

The same	Sodium glutamate		Kajiwara, Samori, 1945 Vinint, 1958
" "	Sodium alginate		Richards, 1955
" "	Ammonium alginate		Hammond, 1958
Liquid filters	Liquid media (meat bouillon, peptone, physiological solution, water, etc.)	Glass liquid bacteria traps (impingers)	Pasteur, 1861 Emmerich, 1880 Miquel, 1882 Straus, Wurtz, 1888
The same	The same	The same	Rettger, 1910
" "	" "	" "	P. P. D'yakonov, 1925
" "	" "	" "	Rousbery, 1947
" "	" "	" "	Henderson, 1952
The same	The same	The same	May, Druett, 1953
" "	" "	" "	Tylor, Shipe, 1959
" "	" "	" "	Groselaude, Herimer, 1961
Liquid foam filters	Saponin, peptone, gelatin, albumen, etc.		Ye. P. Sinel'nikova, 1963

Table 10 (continued)

Vapor or atomized liquid	Liquid nutrient media, (bouillon, peptone, etc.)	Siphon-type bacterial trap	Moulton, 1943
	The same	The same	S. S. Rechmenskiy, 1951
	" "	" "	N. M. Rudenko, 1956

1. Classification of Methods for Collecting Microorganisms from Air

From among the most widely known classifications of methods for collecting microorganisms from air we should indicate the classifications and surveys by Cunningham (1873), Committee on Apparatus in Aerobiology of the National Scientific Research Council of the USA, 1941, Du Buy, Hollaender, Lackey (1945 S. S. Rechmenskiy (1951), A. I. Burshteyn (1954), Albrecht (1957), Tylor and Shipe (1959), V. V. Skvortsov, V. S. Kiktenko (1960), and others.

Table 10 gives the classification of instrumentation and bacterial traps with consideration of the most recent data.

In accordance with the classifications presented in Table 10, we will carry out a detailed description of bacterial traps, give a comparative evaluation of their effectiveness, and present information of the possibility of using a number of instruments for not only microbiological, but also virological investigation of air.

2. Instruments Whose Collecting Mechanism Is Based on the Principle of Deposition of the Dispersed Phase

a) Deposition of Bacterial Aerosols by Gravity

One should regard as the simplest method of detecting microorganisms in air the application of the free settling of aerosol particles containing bacteria or viruses onto glass plates covered with various sticky substances or directly onto a nutrient medium.

Thus, Salisbury (1866) used object glasses to detect particles containing bacteria; he exposed the glasses over night and then examined them under a microscope.

Pasteur (1860) was the first to use the method of gravitational deposition of bacterial aerosols, with settling of the bacteria occurring directly onto a nutrient medium. His investigations were carried out by means of special bottles 250-300 ml in capacity. Pasteur utilized the following procedure: he poured 150 ml 10% sugar bouillon containing a small quantity of brewing yeast into the bottle. After sterilization of the bouillon the neck of the bottle was quickly filled up and it was ready for use. To study air up to 20 bottles were normally used. During sampling of the air the ends of the necks of each bottle were exposed and after the vacuum was filled with air to be tested, they were sealed again. Then all the bottles were placed in a thermostat. Upon termination of incubation a number of bottles were noted in which the medium was discolored by the development of microorganisms in it. Pasteur observed that when 20 bottles were opened in the yard of the Paris Observatory, development of microorganisms was detected in all 20. Of 20 bottles exposed in mountains at an altitude of 2000 m the development of microorganisms was observed in only one. Using these bottles Pasteur carried out a comparative study of the bacterial contamination of the air. Fodor (1881) carried out a large number of experiments in Budapest on bacterial study of the air. The method for studying bacterial seeding of air proposed in 1881 by Fodor provided the use of a solid medium made from fish glue. Bacteria which settled onto the nutrient medium were grown at room temperature. Koch (1881) proposed an original method for studying bacterial seeding of the air. The method method consists in the following: a glass cup 5-5.5 cm in diameter with a wall height of 1 cm is placed on the bottom of a glass cylinder 18 cm high and 6 cm in diameter. The cylinder is tightly closed with a cotton-wool

plug and sterilized by hot air at 150°. Before the experiment the cup was filled with gelatin (agar). Koch left the cylinder with the cup exposed for 5 hours or more, with a shorter period being used in contaminated air. Upon termination of the experiment the cylinder was plugged with cotton wool and placed in a thermostat (Fig. 9).

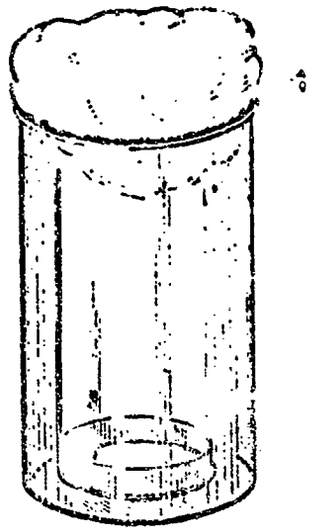

Fig. 9. Sampling air by the Koch method.

After the appearance of the Petri dish the Koch method was not applied in its original form. At present a variation of the Koch method is used: open Petri dishes with meat-peptone agar are placed in the open atmosphere for various periods of time (5-50 min). After seeding of the medium surface by microflora the cups are placed in a thermostat. The sensitivity of this method in collecting pathogenic microflora can be increased by using selective nutrient media. It is not possible to carry out quantitative calculation of microflora by this method.

The Koch dish method was altered somewhat by K. P. Koval'kovskiy (1885): using a wooden cylindrical plug-piston he drew the air out of a cylinder with a capacity of 1 liter. A cup with nutrient medium was placed on the bottom of this cylinder. During the experiment the piston was removed from the cylinder, which was thus filled with air. The microflora contained in 1 *l* of air filling the cylinder settle out onto the surface of the nutrient medium. Thus it is possible to carry out a quantitative count of the microflora.

Clark (1912) proposed that sterile Petri dishes without nutrient medium be set up in the area where the air was to be tested. After 15 min of exposure the dishes are transferred to the laboratory and covered with agar melted and cooled to 40-45°.

I. Ye. Minkevich (1940) recommended using the modified Koch method proposed by Clark in the winter, since when dishes filled with medium are set out the medium will freeze.

During quantitative determination of microorganisms in air by the dish method, V. L. Omelyanskiy (1941) proceeded on the assumption that the quantity of microorganisms contained in 10 ℓ of air could successively be seeded onto a dish surface 100 cm^2 in area in the course of 5 min. However, in later years some investigators showed that microorganisms settle out not from 10 ℓ, but from 3 ℓ of air (A. I. Shafir and P. A. Kouzov, 1948; R. G. Gogoberidze, 1953; Spurny, Jech, Sedlacel, Storch, 1964).

N. N. Pokrovskiy and Ya. G. Kishko (1957), K. I. Turzhetskiy (1957), and others arrived at the conclusion that during studying of bacterial seeding of atmospheric air by the deposition method the formula for calculating the quantity of microorganisms per unit volume is in general inapplicable, since it leads to overstated readings. These authors proposed calculation of the results of studies by the cup method on the basis of counting the absolute number of colonies growing on the dishes. A similar opinion is supported by Yu. A. Krotov (1951) and V. V. Vlodavets (1959), who consider that the dish method is unsuitable for the investigation of, first of all, the air of the open atmosphere, since constantly existing currents of air artificially increase the number of microorganisms deposited on the dishes.

One variation of the method of collecting microorganisms from air on the basis of free settling of aerosol particles from a certain volume of air is represented by instruments proposed by Alvarez, and Castro (1952) and by S. I. Kudryavtsev and coworkers (1966). The instrument designed by Alvarez and Castro is a small cup whose walls are fastened to loops and whose bottom contains a depression for a Petri dish or an object glass. At the moment when the air sample is taken the walls are raised upward and air passes freely through the box. Then the walls are lowered again and the process of settling of aerosol particles onto the slide or Petri dish proceeds. A drawback of the instrument is a fact that the sample is taken from a small volume of air and there is no consideration of microorganisms deposited on the walls of the instrument due to convection and diffusion (Green, Lane, 1957).

The instrument proposed by S. I. Kudryavtsev, A. F. Turov, and N. I. Tonkopiy (Fig. 10) makes it possible to determine the concentration of bacterial particles both under conditions of relative immobility of the air and in turbulent air of the outdoor atmosphere. The instrument consists of a rectangular tank 1 l in volume with four sockets for cassettes which make up its side walls, a hollow cylinder-piston, five cassette-cups installed in sockets of the tank, and a mechanism for automatic lowering of the tank and opening of the lower cassette. Particles enter the closed volume of the instrument at the moment of sampling and settle out onto the nutrient medium of the cassettes.

The Koch dish method and, in general, instruments whose collecting mechanism is based on the principle of settling of bacterial aerosols out of open atmosphere by gravity possess substantial drawbacks. When these methods are used it is impossible to judge the quantitative content of bacteria in a certain volume of air. Calculation methods proposed by various authors (V. L. Omelyanskiy and others) have only relative value

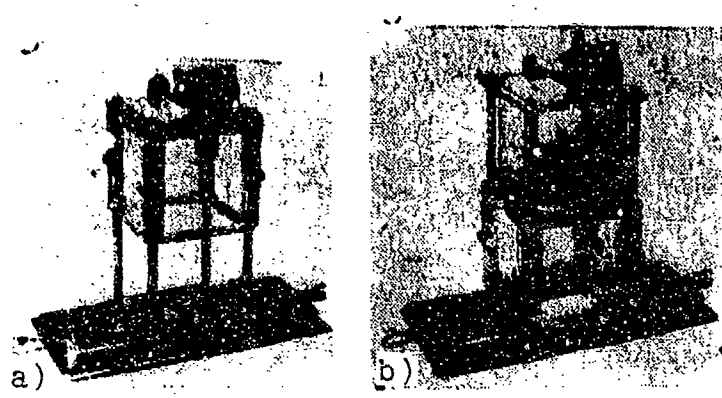

Fig. 10. Aerosol trap designed by S. I. Kudryavtsev, A. F. Turov and N. I. Tonkopiy.
a) instrument ready for sampling; b) instrument after sampling; settling out of bacterial aerosol particles onto the surface of the nutrient medium is underway.

and cannot give a correct answer to the question of the magnitude of bacterial contamination of the air, since the weight and size of bacterial particles and also the air currents will have an enormous influence on the quantity of deposited bacteria. In using sedimentation methods it is also impossible to draw a correct conclusion concerning the qualitative relationship among microorganisms in the air, since the microorganisms deposited on the Petri dish are basically those connected with large particles of dust. An essential deficiency of this method is the great duration of time required for sampling; because of this the method can be used for determining concentration of solid or liquid aerosol particles with a very low evaporation rate.

b) Deposition of Bacterial Aerosols by the Impact Action of an Air Jet (Inertial Deposition)

Owing to their low mass, particles of bacterial aerosols tend to remain suspended in air for a prolonged time. Therefore the deposition of particles makes it advisable to use methods based on adhesion of particles to the surface of nutrient media

by means of the impact effect of an air jet. In this case it is necessary to meet a number of conditions: excessively slow passage of the aerosol through the instrument prevents detection of highly dispersed fractions of the aerosol, while excessively high speeds of flow leads to "skipping" of aerosol particles.

The general principle of this method of collecting aerosol particles consists in directing the flow of air against the nutrient medium or against a plate smeared with sticky neutral substances. After obtaining kinetic energy, the particles, as a result of inertia, collide with the surface of the nutrient medium and adhere to it. The method of collecting bacterial particles (spores) from an air flow on glass plates has found wide acceptance (Scheppegrell, 1922; Durham, 1944; Hyde and Williams 1943, and others).

Thus, Scheppegrell used ordinary object glasses 76 × 25 mm in size smeared with a gluey coating in the form of a mixture of gelatin with vaseline. The plates were shielded for 24 hours. This method is extremely simple and cheap and provides the possibility of continuous work. However, as a quantitative method of evaluating bacterial seeding of the air under field conditions it has serious drawbacks due to the presence of an edge effect and to turbulent deposition, and also to predominate deposition of large particles only.

When the cup method is used in field conditions the same difficulties are encountered as during application of plates - problems due both to the rate of deposition of aerosol particles and to wind speed, as well as the aerodynamic effect caused by the aerodynamic "shadow" from the edge of the cup.

Gregory and Stedman (1953) made a careful study of the effectiveness of collecting fungus spores by mea of Petri

dishes. A Petri dish with 15 cm^3 of agar was set in the horizontal position in a wind tunnel. The authors determined the average capture coefficient per cm^2 of surface. A narrow band of deposited spores was observed behind the rim of the dish facing in the direction of the oncoming flow and also in front of the back rim of the dish. At an air flow speed of 0.5 m/s the capture coefficient was not particularly great, but at 1.1 and 1.7 m/s a substantially greater quantity of particles was detected at both the front and back rims of the dish. At a wind speed of 3.2 m/s and more the aerodynamic "shadow" was observed most clearly, since the rims of the dish (1 cm high) screened almost the entire surface of the agar. The effect created by the rim of the Petri dish can be eliminated when the dish is placed on the bottom of a metallic cylinder which is installed so that the upper edge of the cylinder is set flush with the axis of the wind tunnel.

When the Petri dish was placed in the vertical position and the wind speed is 9.4 and 5.5 m/s the deposition of particles in a central zone 2.5 cm in radius was 4 times greater than in the annular zone 1 cm wide along the rim of the dish. A wind speed of 3.2 m/s created conditions under which the quantity of spores was 75% greater around the rim of the cup than on the remaining portion of the dish bottom.

The method of deposition in open atmosphere was used by Frankland (1887), Richards (1955), Werff (1958) and others.

Deposition of particles on plates smeared with sticky substances [vaseline, gelatin/glycerin, a mixture of vaseline with paraffin (12.5%), etc.], was carried out in instruments of the aeroscope or aerokoniscope type by Salisbury (1866), Maddox (1870), Airy (1874), Cunningham (1873), Cristoff (1934), and Shitikova-Rusanova (cited in K. M. Stepanov, 1935). The oeprating principle of these instruments was based on deposition

of aerosol particles under the action of an aerodynamic wind pressure. The Cunningham aerokoniscope was the most highly perfected in design. The Cunningham aeroscope is shown on Fig. 11. The instrument consists of a conical funnel equipped with fletching, which turns it to point in the direction of the wind. Aerosol particles were deposited on a sticky cover glass located behind the output opening of the funnel.

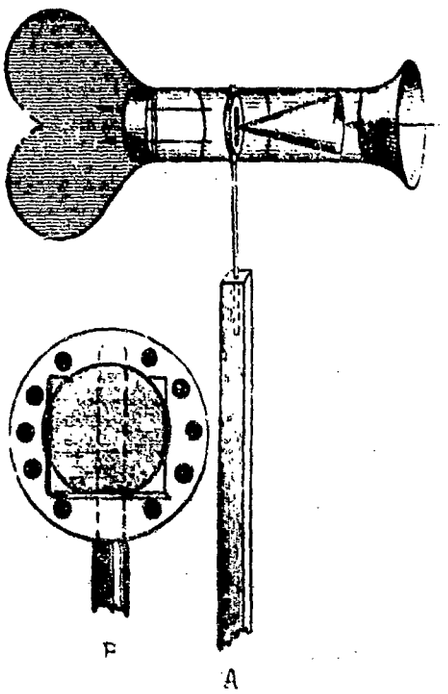

Fig. 11. Cunningham aeroscope. A - side view of the apparatus (partly sectioned); B - sticky cover glass set behind the outlet of the funnel.

Miquel (1895) also made successful use of the aerokoniscope. Bacterial particles were deposited on an object glass coated with glycerin. The glass was arranged 2-3 mm from the opening in the instrument; opening diameter was 0.5-0.75 mm. Thanks to this structure the effectiveness of the instrument was substantially higher than that of the Cunningham aeroscope. Thus, the Miquel

instrument collected approximately 100 times more particles than the Maddox and Cunningham aerokoniscopes.

Such instruments as the May cascade impactor, the Herst automatic trap, and the Gregory portable trap have also been used for bacterial study of the air. Descriptions of the instruments and their operating principles are given in Chapter II.

Thus, Herst carried out a comparative study of the effectiveness of his instrument (trap) with deposition of spores on horizontal plates and on vertical cylinders. The studies were carried out under open-air conditions. It was found that spores less than 20 μm in size were detected in quantities 25 times greater on the average in the Herst trap than on object glasses. Virtually no extremely fine spores were found on horizontal glasses or on vertical cylinders.

Gregory, Hamilton, Sreeramulu (1955), Hyde (1959) and others used the Herst trap in their observations.

However, in the long run instruments of the konimeter type do not meet the requirements of aerobiology: they are suitable only for measurements of high concentrations, since the volume of aspirated air is small and these instruments cannot be used with cultural methods of analysis, since sampling is carried out on glass plates. The aerodynamic properties of the majority of instruments are such that they exclude the possibility of isokinetic sampling of the air (with the exception of the May and Herst instruments); deposition of particles of a biological aerosol occurs at a high speed, which not only breaks up particle aggregates but also causes the death of the bacteria themselves.

Evaluations of the effectiveness of these instruments were carried out by Davies et al. (1951) and by Green and Lane (1957).

Instruments of the aerokoniscope type could be used only for qualitative analysis of the bacterial seeding of the air, since their capture coefficient depends on the pressure head of the air and the detection of one or another microorganism merely indicates that the given organism is actually existing in the air, without any possibility of its quantitative determination (Gregory, 1961). The operating principle of aerokoniscopes was used subsequently by a number of authors to create instruments which collect microorganisms directly on a nutrient medium.

The first instrument used according to the indicated principle was the Pushe aeroscope (1859) (cited by F. Gregory, 1964). The structural principle of this instrument was subsequently used to create instruments of a similar type. Thus, this principle was the basis for designs of instruments by Pavlovskiy (1885), N. Keldysh (1886), Hesse (1884), and others.

A. Pavlovskiy proposed using a bent glass tube whose inner surface was coated with a thin layer of gelatin. Air was passed through the tube. The bent form of the tube facilitated higher retention of microorganisms from the aspirated volume of air. The instrument has a number of essential drawbacks: counting the quantity of microorganisms (colonies) which grow on the gelatin layer in the glass tube is hampered by the impossibility of distinguishing them clearly; in addition, with incidence of microorganisms which thin the gelatin the latter slips in the joints of the tube.

N. Keldysh passed air through a straight glass tube whose inner surface was coated with nutrient medium. After passage of the air the tube was closed at both ends. However, even in a straight horizontal tube there was nonuniform distribution of microorganism colonies - the major mass of them was concentrated at the inlet opening.

The Hesse instrument consists of a cylindrical glass tube 70 cm long covered at both ends with rubber plugs. The tube is fastened to a wooden stand and connected successively to two flasks. Water is poured into one flask, while the other remains free. With passage of water from one flask to the other a rarified space is created and air is drawn into the glass tube. Nutrient medium is applied to the inner wall of the tube. Thus the microflora contained on aerosol particles are brought into contact with the surface of the nutrient medium. Hesse noted that colonies of bacteria developed mainly at the inlet to the tube, while mold fungi penetrated much further into the tube. On this basis Hesse made the assumption that spores of fungi are lighter than bacterial nuclei and apparently are present in the air as single individuals. At the same time the bacteria in the air may be in the form of large accumulations (aggregates) or they may be contained on large particles of dust.

The Hesse method was used by Frankland (1887) and by Hart (1887). Frankland noted that the colonies in the tube showed the greatest growth when the tube was turned with the inlet opening facing the wind. Therefore studies were carried out with the tube arranged at an angle of 135° to the wind direction. These experiments established the importance of taking into account aerodynamic factors in studying the bacterial seeding of air.

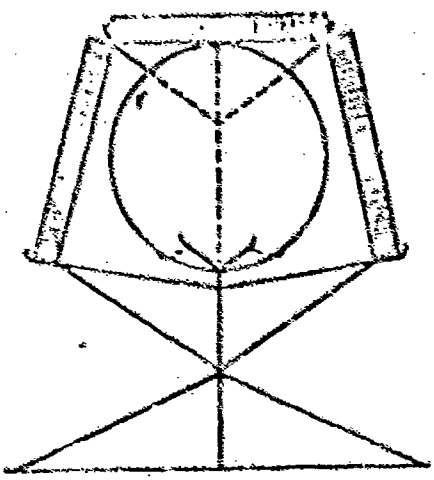

Fig. 12. Mosveyev deposition plate.

The aeromicrobiological fixer - the Matveyev deposition plate (1951) - is based on bringing bacteria to the nutrient medium in a flow of air. The instrument consists of a light wire frame with sockets for dishes (Fig. 12).

The dishes are placed on the framework with the medium facing out. Four dishes are located vertically with respect to the ground or the floor, while one dish is placed level at the top. Aerosols moving in the vertical direction are deposited on the upper dish, while aerosols moving horizontally and at some angle to the vertical are deposited in the vertically arranged dishes.

V. S. Kiktenko and coworkers proposed a modification of the Matveyev instrument. Petri dishes were placed in special metallic sockets which could be set in any position, with the setting dependent on the direction of air movement. The instrument is equipped with a vane (Fig. 13).

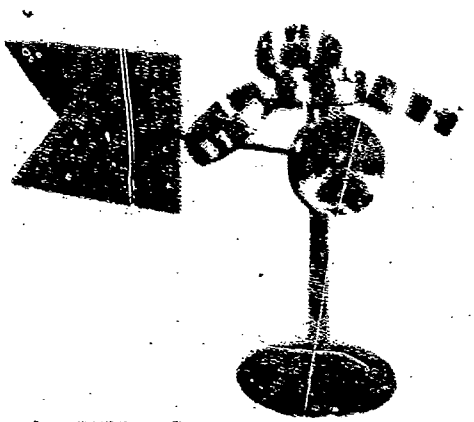

Fig. 13. Modification of the Matveyev instrument.

A number of authors (F. A. D'yachenko, G. V. Savchenko, and L. F. Permogil'skaya, 1957) determined a substantially greater number of microbes in the air with the Matveyev instrument than with the instrument developed by D'yakonov and Krotov.

In the opinion of Yu. A. Krotov, a constant stream of air under open-air conditions artificially increases the number of microorganisms deposited on the dishes; this is the basis for the possible comparatively greater effectiveness of the Matveyev instrument.

The instrument designed by Stoyanovskiy and Rev (1954) is based on a different principle. Air impacts against the surface of the nutrient medium (poured into a Petri dish) during movement of a motor vehicle. The instrument (Fig. 14) consists of a flared tube (1) which captures air, a conducting tube (2) and head (3), containing a Petri dish with nutrient medium.

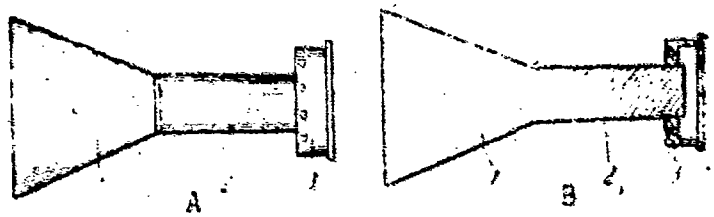

Fig. 14. The Stoyanovskiy-Rev instrument (see description in text).
A - general view; B - section along the axis.

The flow of air is captured by the flared tube, passes through the guide tube, where it takes on greater velocity, and impacts forcefully against the surface of the nutrient medium in the Petri dish; this ensures that particles suspended in the air will adhere to the medium.

Results of investigations using the Stoyanovskiy-Rev instrument are substantially influenced by the rate of travel of the vehicle, which in turn determines the effectiveness of the bacterial trap.

In the Vas'il'yev instrument (1957) the flow of air is directed to the nutrient medium by a fan. The instrument consists of

three parts: a conical housing, the fan, and a support. The four-bladed fan is placed in the middle of the instrument housing and is activated manually (Fig. 15).

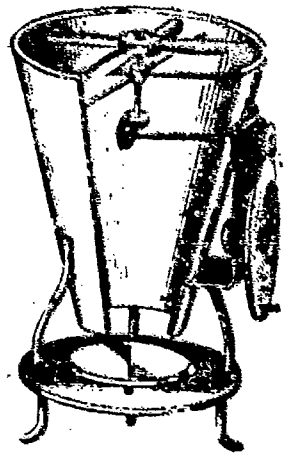

Fig. 15. Vasil'yev instrument.

A. Ye. Vershigor (1959) considers that this instrument is awkward and does not permit consideration of the quantity of air passed through it.

L. I. Trashchenko recommends investigation of bacterial seeding of the air by means of a vacuum cleaner. A Petri dish is set in the vacuum dust bag. In his opinion the effectiveness of the instrument is at least equal to that of the Krotov instrument.

The Kishko instrument (1959) is based on the impact action of a stream of air. The instrument is intended for studying the bacterial seeding of air from aircraft and consists of a collector, cassettes with Petri dishes, and an air flow meter (Fig. 16).

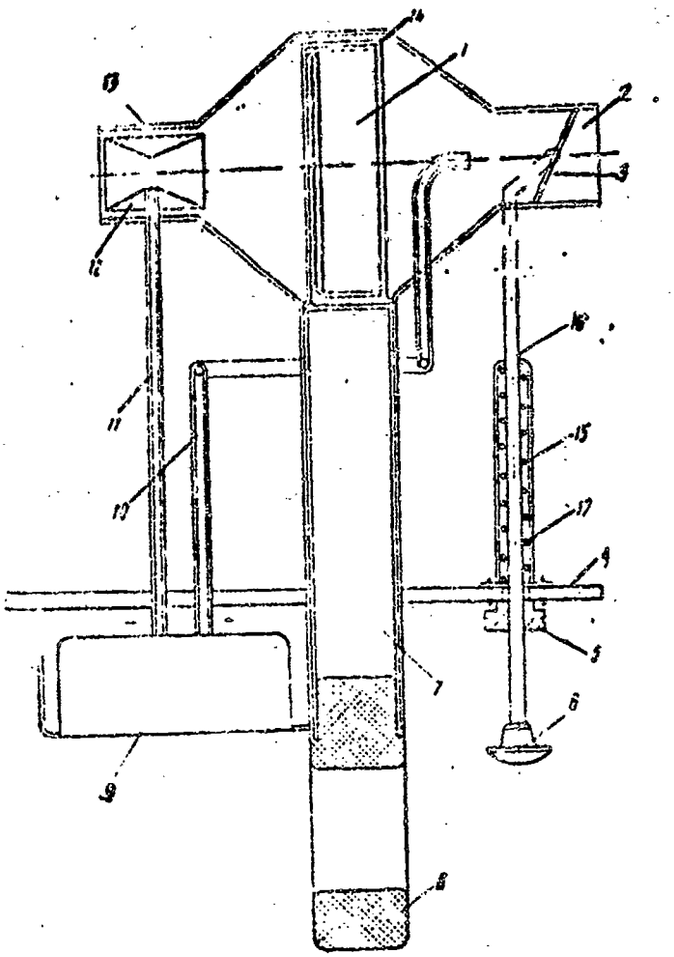

Fig. 16. Mishko instrument (diagram) for sampling air from an aircraft (see description in text).

The collector has a sampler (2) with throttle plate (3), which is opened or closed by means of a series of working parts which are connected to one another (15, 16, 17). Regulation of the air travel speed is accomplished by means of attachments (5, 6). The cassette (7) with the Petri dish in a special socket (14) is moved by a lever (8) into the funnel-shaped opening located behind the sampler. The collector terminates in a contraction and in an air exhaust tube (13) with a Venturi tube (12). The quantity of passed air is fixed by instrument (9).

Air enters the instrument at a speed which depends on the speed of the aircraft (150-180 km/h). Up to 0.5 m^3 of air is passed through the instrument in a time period of 3-6 minutes. The surface of the nutrient medium is coated with a thin layer of presterilized neutral oil MG-10 with a low freezing point (to [sic] 90-100°) to permit successful utilization of the instrument at the low temperatures at high altitudes.

In 1961 V. M. Khil'ko proposed an instrument (Fig. 17) consisting of an electric motor (1) activated by electric current from flashlight batteries, a light bulb (?), and a fan (3). When the motor is switched on air drawn in by the fan impacts against the surface of nutrient medium in a Petri dish, as a result of which particles of the microbe aerosol stick to the medium.

During sampling the instrument can either be worn on a shoulder or chest strap or it can be placed on the ground. The total weight is 700 g. The instrument will process up to 20 *l* of air per minute. Two batteries provide continuous operation for 3-4 hours. As the author notes, the collecting capacity of the instrument is inferior to that of the Krotov unit; however, this instrument differs favorably from the latter in that it does not require a powerful source of electric current and it can be used under field conditions.

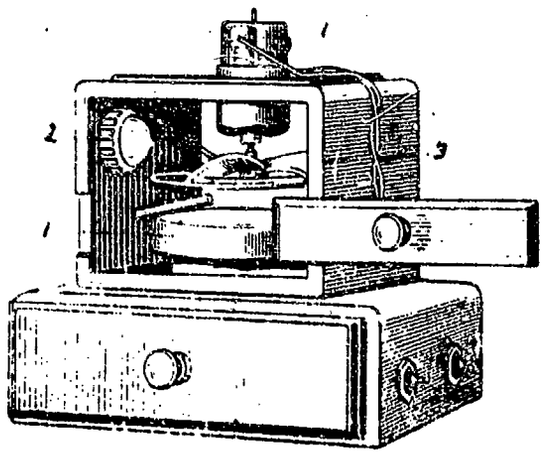

Fig. 17. Khil'ko instrument.

In 1938 Proctor and Parker proposed an instrument in which air is passed through a tube which opens to the outer wall of the cockpit in an aircraft, traveling (during flight) at a rate of 0.3 m^3 per minute to a rotating metallic disk whose surface has six openings. Circles of paper impregnated with specially purified oil are placed in these openings; the paper ensures trapping of airborne microorganisms. Exposure of each disk lasts from one to several minutes, depending on the degree of assumed contamination of the investigated layer of air. According to information from the author the instrument is not capable of collecting all phases of a bacterial aerosol.

In the last 15 years a number of authors have proposed bacterial traps based on the principle of inertial deposition of particles of a bacterial aerosol: centrifuging, the slip method, and the "funnel" and "mesh" methods.

In the instrument designed by Hollaender and Dalla Valle (1939) is based on the "funnel" method; air is directed through the narrow portion of the funnel into the wider end, located directly under a Petri dish with a solid nutrient medium. The rate of arrival of air equals 0.0283 m^3 per minute. According to information from a number of authors (V. V. Vlodavets, 1959; A. I. Zhukova, 1962), the instrument has low sensitivity, since the speed of the air stream and the consequent entrainment force are not great. A significant part of highly dispersed aerosols will not be retained by the agar but carried off to the surface.

Breakup of aerosol particles even prior to impact against the agar surface was realized in the Du Buy and Crisp instrument (1944). The instrument consists of a round housing with an outlet opening for air, a Petri dish, and a brass disk cover with 300 openings. The cover fits tightly over the housing, being attached to it by two cover clamps. Air enters the

instrument through the openings in the cover plate, hits the surface of the agar, travels along the surface of the medium, and is then ejected through the outlet opening, located on the bottom of the case. The presence of a cover with openings ensures more uniform distribution of the flow, which travels under high pressure. At present this method is widely used in studies of aerial propagation of infections in bacteriological and virological laboratories.

Comparing the effectiveness of their instrument with the bacterial trap designed by Hollaender and Dalla Valle, Du Buy and Crisp established that their instrument possesses more than double the collecting capability. Instruments by Wells (1933) and Shafir (1941), as well as others, are based on the principle of utilizing centrifugal force for collecting particles of bacterial aerosols.

The Wells instrument (Fig. 18) consists of a metallic cylinder, and electric motor, and a jacket.

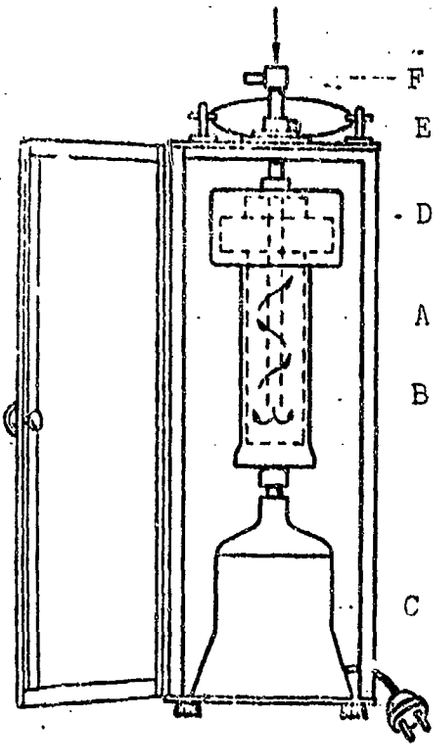

Fig. 18. Wells instrument. A - air guide tube; B - main section of centrifuge (cylindrical cup); C - vertical motor; D - rotor housing; E - terminal; F - fitting connected with a manometric tube.

Centrifugal force directs the stream of air against the surface of the nutrient medium located on the inner wall of the cylindrical cup; thus the medium is seeded by particles containing bacteria. The cylinder is rotated at a speed of 3500-4000 r/min. According to data from the author, the instrument makes it possible to study large volumes of air (30-50 l/min) and to carry out quantitative calculation of the microflora. This instrument is widely used in routine hygiene and bacteriological investigations of the air (Pingus and Stern, 1937).

Bourdillon et al. (1941) indicate the possibility that the Wells instrument traps only 5-50% of highly dispersed particles of a bacterial aerosol.

Using the principle proposed by Wells, A. I. Shafir (1945) also designed a centrifugal instrument. Shafir introduced a number of substantial improvements in his instrument: the centrifuge can operate without electric power supply; air moves upward, which facilitates loading of the instrument, since the fan is located below; the axial air-guide tube is made of glass and is easily removed and sterilized. For more exact analysis and counting of colonies growing on the cylinder walls a small mirror is introduced inside it, reflecting light from an illuminator. A diagram of the Shafir instrument is shown on Fig. 19.

The instrument is constructed as follows: metallic cup (3), rotating at 3000 r/min, is fastened to the shaft of motor (5). Before operation a sterile cylinder with melted nutrient medium (2) is placed in this cup. The snail of the centrifugal fan (8) with a rotor having 12 blades is placed under the base of the metallic cup. During motor operation the fan aspirates air out of the space bounded by the removable airtight jacket (4), with new volumes of air replacing that removed in the instrument by entering through openings in air-guide tube (1). The quantity

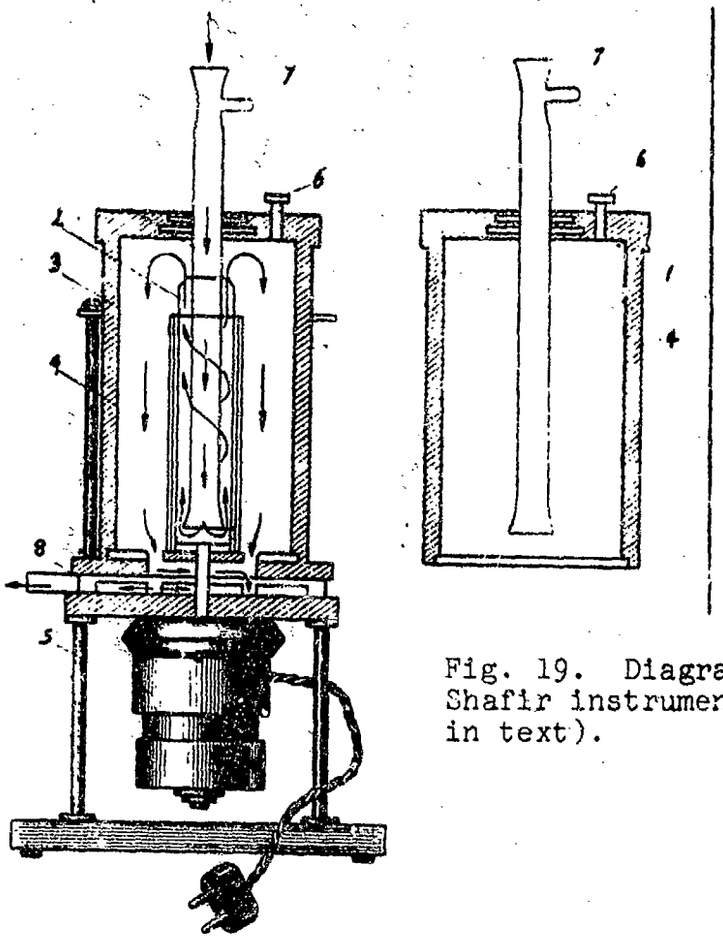

Fig. 19. Diagram of the Shafir instrument (see description in text).

of air is measured by a micromanometer connected to fitting (7) by a rubber tube.

According to data from Yu. A. Krotov (1953), the Shafir centrifuge captures up to 42.9% of the number of microorganisms which can be detected with a slit instrument. P. F. Milyavskaya (1947) considers that the Shafir centrifuge is substantially inferior to aspiration methods. The reason for the divergence may consist in the fact that on the walls of the centrifuge cylinder the colonies were grown not from single cells but from several, which lowered the results of the investigation during use of the centrifuge. Later A. I. Shafir replaced the agar with 20 ml of a buffer solution in order to carry out virological studies.

Evaluation of the effectiveness of the Wells and Shafir centrifuges by Soviet and foreign investigators agrees with the theoretical calculations, according to which only particles with a radius several times greater than the size of the average dimensions of bacteria will be deposited on the surface of the nutrient medium in these instruments. N. A. Fuks (1955), working on the basis of the theory of deposition of aerosols by means of centrifugal force, came to the conclusion that a centrifuge rotating at ordinary engineering speeds is suitable for deposition of particles 1-2 μm in size. The deposition of bacterial and viral aerosols with dimensions of fractions of a micron requires a supercentrifuge rotating at a speed of several hundred rotations per second.

It was established that the capture coefficient comprises 100% for particles 2.3 μm in diameter, but only 50% for particles with a diameter of 0.77 μm. The capture coefficient is lowered with a reduction in particle size. In practice the Wells centrifuge can detect no more than 69% of the microorganisms found in the air (Phelps, Buchbinder, 1941).

The collecting capacity of a centrifuge is not proportional to the r/min. Thus, B. V. Boykov (1954) established that an increase in the r/min of the cylinder of the Shafir centrifuge substantially reduces its collecting effectiveness. The optimum passage of air into the centrifuge should not exceed 15 *l* per minute. At a rate of 40 *l* per minute the number of microorganisms collected is reduced by a factor of 10, since a substantial portion of the aerosol particles pass along the nutrient medium without sticking to it. To increase the effectiveness of particle collection, Boykov recommended replacing the agar with water; he explains this by the fact that water is better able to absorb particles suspended in air and, in addition, it is clear that in a moist medium large particles are broken up into smaller ones.

The Rechmenskiy aerocentrifuge (Fig. 20) is based on the principle of aspiration of air and attaching action (1951).

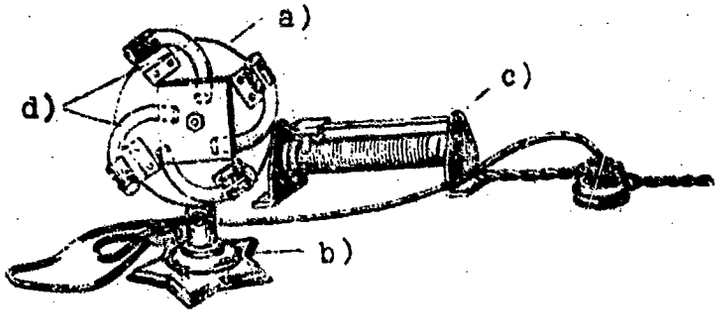

Fig. 20. Rechmenskiy aerocentrifuge (see description in text).

The instrument consists of a metallic disc (a) fastened to the axis of an air fan (b). The disc has four clamps for glass cylinders. The cylinders contain cellophane plates smeared with highly refined oil. During rotation of the disk there is vigorous pressing of the air into the cylinders, with microorganisms sticking to the surface of the cellophane plates. Seeding is accomplished by pressing the plates against the surface of the nutrient medium. A supply of sterile cylinders makes it possible to take a substantial number of samples in air. However, the Rechmenskiy centrifuge does not allow quantitative calculation of microorganisms in the air.

Among the general drawbacks of instruments based on the centrifuge method the following should be pointed out: the nutrient medium is usually nonuniformly distributed over the inner surface of the cylinder and during rotation it is spread further, hampering calculation of the quantity of colonies growing on the surface of the nutrient medium because of its opacity; the instruments collect mainly the large-drop phase of bacterial aeroplankton. Besides this, it should also be pointed out that the Wells and Shafir instruments are bulky in construction and expensive.

At present the most widely used instruments are those based on the slit principle. The variety of models of these instruments continues to expand without end.

This method of collecting bacterial aerosols was first proposed in England in 1941 by Bourdillon, Lidwell and Thomas. Later the instrument was improved by these same authors (1942). In the USSR the Krotov apparatus was designed on the slit principle. In Poland an instrument of this type was designed by Lazowski and Kancelarczik (1956); in Romania by Adrelean, Etingher, Janistea, Barnea, Goldner (1954); in the USA by Luckiesh et al. (1946), and Decker and Wilson (1954) and Pady (1954); in Czechoslovakia by Raska and Sip (1949) and Symon et al. (1956); and in Sweden by Laurell, Löfström, Mangusson (1947).

In inertial instruments the aerosol passes at various controllable speeds through a plain slit or a round opening; emerging from it, it strikes the surface of a nutrient medium with considerable force. As the air stream impacts on the surface the particles contained in it are deposited by inertia on the surface of the nutrient medium. The profile of the instrument slit and the speed of the aspirated air play a major role in the effectiveness of capture of particles of bacterial and viral aerosols. A characteristic feature of slit instruments is the fact that during impact of solid particles 5-10 µm and larger in size against the solid surface they are broken up, which facilitates establishing more exact readings of the bacterial loading of the air (V. A. Fuks, 1955).

The instrument designed by Bourdillon, Lidwell and Thomas (1942) is a cylinder closed from above by a cover equipped with a sector-shaped slot. A Petri dish with medium is placed under the cover. An electric motor draws air into the instrument through the slit in the cover and ensures uniform rotation of the dish.

Passing through the slot, air impacts against the surface of the nutrient medium at high velocity and seeds it with microflora. The apparatus is driven manually or mechanically. Air is passed through the slit at a rate of 27 l/min.

Thomas (1955) showed that the percentage of capture of microorganisms from the air depends mainly on the size of the aerosol particles. Up to 70% of particles 2 μm in diameter are captured, while only 15-20% of smaller ones are trapped.

The instrument developed by Bourdillon et al. has not found wide acceptance, despite favorable comments on its operation by a number of investigators. The complexity of the design, the low effectiveness in respect to highly dispersed bacterial aerosols, and operational deficiencies are factors which have prevented its entry into the practice of hygiene and bacteriological research.

Somewhat later Yu. A. Krotov (1953) proposed an instrument operating on the same principle as the Bourdillon instrument, but with some improvements. The Krotov apparatus (Fig. 22) is a cylinder closed at the top by a removable cap, under which a Petri dish with nutrient medium is installed on a platform which is rotated by the turbulent flow of air. Inside the instrument there is an electric motor with a centrifugal high-pressure fan, ensuring intake of air and rotation of the table containing the Petri dish (Figs. 21 and 22).

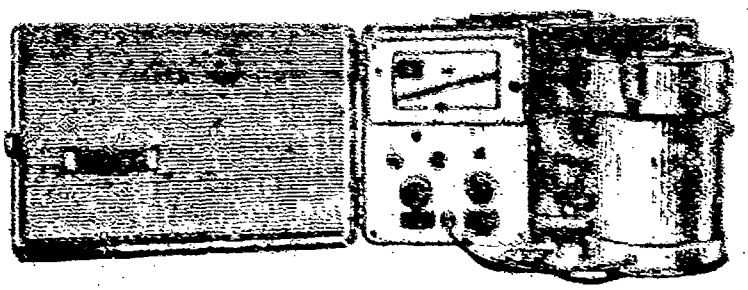

Fig. 21. Krotov instrument (external view).

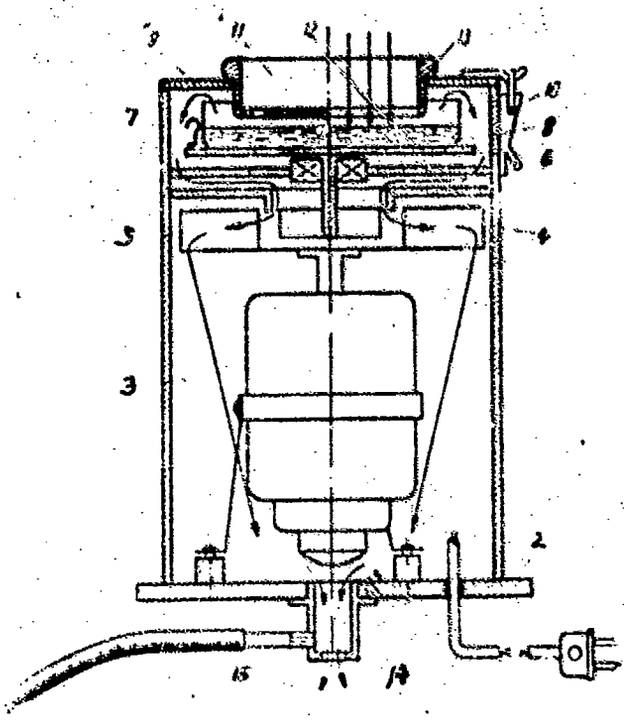

Fig. 22. Diagram of the Krotov instrument. 1 - cylindrical housing; 2 - base of housing; 3 - electric motor; 4 - centrifugal fan; 5 - eight-bladed wheel; 6 - disc; 7 - spring; 8 - Petri dish; 9 - instrument cover; 10 - snap-down clamps; 11 - plexiglas disc; 12 - wedge-shaped slit; 13 - split ring; 14 - fitting with diaphragm; 15 - outlet tube.

Air enters the instrument through a wedge-shaped slit located along the radius of the Petri dish. Passing through the slit with high linear velocity, the air impacts against the surface of the nutrient medium in the Petri dish; microorganisms suspended in the air are deposited on this medium. The table with the Petri dish rotates at a speed of 60-100 r/min. The instrument shows optimum collecting capacity during passage of 25 l of air per minute. The stream of air travels at a speed of 15-20 m/s. Aspiration rate is regulated by means of a rheostat. Alternating current from a 127 and 220 V net is used as the motor power supply.

During preparation of the instrument for operation standard Petri dishes 10 cm in diameter are selected and prefilled with the required nutrient medium, in a quantity no greater than 15 ml. The amount of nutrient medium on the dish is of critical significance, since if there is too much the motor is unable to rotate the table at the required speed; besides this, it is necessary to consider that most complete trapping of microorganisms is ensured with a distance of 2 mm between the cup and the slit.

The Krotov instrument has a number of essential drawbacks: the apparatus operates only where electric power is available and it cannot be used to collect viruses and rickettsia, which do not grow on solid nutrient media. Since the instrument is not adapted for isokinetic sampling of an aerosol, it cannot be adequately effective in studying bacterial contamination of the air of the outdoor atmosphere, where there are frequent changes in the direction and velocity of air motion.

Comparative experiments on evaluating the effectiveness of this instrument, carried out by the author himself, showed high sensitivity as compared with certain other bacterial traps. Designating the effectiveness of his own instrument at an arbitrary 100%, Yu. A. Krotov established that the collecting capacity of the Shafir instrument equals 42.9%; that of the D'yakonov instrument 40.9%, and the fish method, 30.9%.

At present the instrument has been somewhat improved, and the "Krasnogvardeyets" plant is issuing a new portable model of the apparatus.

G. I. Sidorenko (1956) found the best collecting properties to be those of the Rechmenskiy instrument, which was compared with the Krotov instrument and with membrane filters. V. V. Vlodavets (1957), taking readings of the Krotov instrument as 100%, found that the best collecting qualities with respect to

bacterial aerosols are those of the Rechmenskiy instrument (139%), while membrane filters No. 4 collect only 82.1%, electrostatic precipitators 62.8%, the D'yakonov instrument 59.9%, the Shafir unit 37.7%, the Koch method 35.4%, and the Zubarev instrument, 21.7%. After carrying out 1500 analyses of atmospheric air using membrane filters, the dish method, and the Krotov instrument, V. I. Bugrova (1957) concluded that the Krotov apparatus can be used also for investigation of bacterial contamination of atmospheric air. However, S. I. Kudryavtsev and N. I. Tonkopiy observed that with air moving at 0.5 to 4.5 m/s the effectiveness of the Krotov instrument in capturing particles 3 to 35 μm in diameter is quite low. Thus, up to 75-80% of particles with a dispersion of 3 to 8 μm were not retained by the instrument, while a collection of particles 20-35 μm in size subjected to insignificant capture in the aspiration process were collected only within the limits 5.6-8.2%.

Table 11 gives generalized data which characterize the comparative effectiveness of different instruments, mainly with respect to the Krotov apparatus, for which the collection capacity is taken as 100%.

In connection with the fact that certain instruments based on the slit method possess fairly high effectiveness, modifications of instruments of this type have appeared recently. Thus, Schuster (1948) constructed an instrument designed for automatic sampling. The last model of his instrument is extremely portable and is designed for prolonged sampling. Kuehne and Decker (1957) studied the effectiveness of Schuster instrument and found that the apparatus has a number of advantages as compared with existing standard specimens of American instruments.

In 1948 Lidwell mounted two rotating dishes with solid nutrient medium in a single air-tight self-contained box; two slot openings were located above the dishes. This modification

Table 11. Comparative effectiveness of different bacterial traps according to data from a number of authors.

Designers of bacterial traps	Effectiveness of the traps, %							
	Krotov apparatus	Shafir centrifuge	Electrostatic precipitator	Zubarev instrument	Membrane filters	Rechmenskiy instrument	D'yakonov Instrument	Kiktenko instrument (cotton-wool filters with impregnant)
Yu. A. Krotov, 1953	100	92.9	—	—	—	—	60.4	—
V. A. Zubarev, 1954	—	80	—	100	—	—	—	—
G. I. Karpukhin, 1952	100	46.3	—	—	—	—	64.2	—
V. V. Vladavets, 1957	100	39.7	62.8	—	82.1	139	59.9	—
A. Ye. Vershigora, 1958	100	—	130	—	—	—	—	—
A. Ye. Vershigora, 1958	— 130	— —	157 142	— —	— —	150 150	100 100	— —
V. V. Vladavets, Ye. Yu. Zuykova, M. A. Mosova, 1958	100	51.1	73.2	36.6	97.3	—	—	—
V. S. Kiktenko, 1961	—	—	—	22.2	—	—	100	213

of the slit instrument somewhat increased sampling time. A similar improvement of the slit instrument was carried out by Zampach (1959). The instrument consists of a case, inside of which a drum with two rows of plastic strips coated with a two-millimeter layer of nutrient medium rotates slowly. The rate of drum rotation is one complete turn in 12 hours or more. Air enters the instrument through narrow wedge-shaped openings located above the strips. The current of air is created by a six-bladed wheel connected to an electric motor. After sampling the strip is placed in a small bakelite tank in the thermostat. Colonies are counted after 48 hours.

In 1962 A. Andersen and M. Andersen proposed the "Monitor" instrument for prolong sampling. The instrument not only makes it possible to carry out investigations of the air over a significant time interval, but also to determine bacterial contamination of air at a given moment in the sampling process. The instrument consists of a rotating drum covered with a layer of agar. The drum can be rotated at different speeds ranging from 1 r/min to 1 r/h; speed is set according to the purpose of the study and also according to the assumed magnitude of bacterial seeding of the air. The stream of air passes through a slit and impacts against the surface of the rotating agar, with aerosol particles being deposited on the latter. In all the line of impact extends for 14 meters 30 cm. With every complete rotation the drum is shifted downward by 3 mm. The instrument is equipped with a heating element which makes it possible to use it as a thermostat to grow microorganisms deposited on the nutrient medium (Figs. 23, 24).

The designers of this instrument carried out a comparison of the effectiveness of the "Monitor" with the aerosol sampler designed by Andersen (1958). Particles of bacterial aerosol 5 um in size were collected.

The total number of particles collected from 27.8 *l* of air by the "Monitor" amounted to 4911, while the Andersen instrument collected 4683 (Fig. 23).

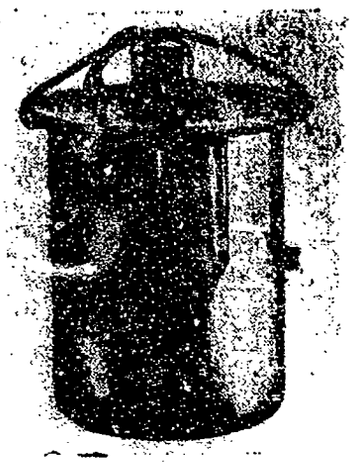

Fig. 23. External view of the "Monitor" instrument.

Fig. 24. Growth of microorganism colonies on the agar surface in the "Monitor" instrument.

In 1956 Lazowski and Kancelarczik developed a general-purpose instrument using both slit and liquid methods of collecting aerosols. The instrument has a unit for sterilization of the output air.

Kuehne and Decker (1957) installed a timer on the portable Decker and Wilson instrument in order to give the dish a controllable rate of revolution: 1, 2, 5, 5, and 12 r/hour.

Like other instruments based on the impact principle, the unit designed by Raska and Sip (1949) is called an "aeroscope." Air enters this instrument through a slot 0.25 × 27.5 mm in size at a rate of 13-15 *l*/min (linear speed 30 *l*/min). In other ways the instrument is virtually no different from other "slit" apparatuses: the aerosol particles strike a nutrient medium in a Petri dish located on a rotating plate. The Petri dish is placed in a chamber of organic glass 105 mm in diameter and 50 mm high. The optimum distance between the slit and the surface of the nutrient medium is controlled and should amount to no more

than 2 mm. The quantity of microorganisms per m^3 of air is calculated by the formula

$$n = \frac{a \cdot 10^3}{v},$$

where v is the volume of air passing through the nozzle, while a is the number of colonies of microorganisms on the Petri dish.

In 1958 A. Andersen developed a cascade dish impactor used to sample bacterial aerosols. In this instrument the "mesh" method is combined with the method of a cascade impactor. The instrument is distinguished by high collecting effectiveness. The apparatus (Figs. 25 and 26) consists of six stages through which air passes in succession. Each stage has a disc with 400 openings and a Petri dish with agar located underneath it. The size of the openings is constant but is reduced for each successive stage. Consequently, the speed of the air flow is successively increased from stage to stage. As a result the largest particles, containing bacteria, impact in the upper dishes, smaller ones stick to the middle dishes, and only highly dispersed fractions of the bacterial aerosol are deposited in the bottom dishes. The instrument is so designed that with passage of air at a rate of 28 *l*/min any particle more than 1 μm in diameter should be retained on one stage or another. Thus, this instrument serves not only to collect microorganisms and determine their quantity, but also for determining the fractional dispersion composition of the particles of bacterial aerosol. The Andersen instrument is widely used in the USA. Thus, during an outbreak of ornithosis in the Portland region (Oregon, USA) this instrument was used for inspection of a meat-processing establishment (Spendlove, 1957), with the result that the source of the infection was identified and measures were proposed to prevent entry of infectious material into the air. In addition, the use of the instrument made it possible to establish just exactly

which fractions of the aerosol carry microorganisms. Consequently, under certain conditions the instrument can be used for virological studies of the air.

Fig. 25.

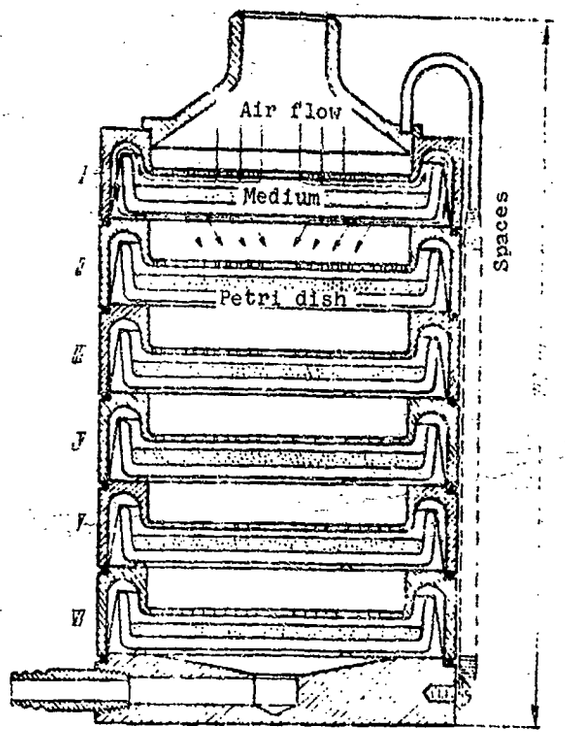

Fig. 26.

Fig. 25. The Andersen cascade dish impactor.

Fig. 26. Diagram of the Andersen apparatus. I - stage No. 1 (1.16 mm; 1.08 m/s); II - stage No. 2 (0.91 mm; 1.80 m/s); III stage No. 3 (0.71 mm 2.95 m/s); IV - stage No. 4 (0.53 mm; 5.26 m/s); V - stage No. 5 (0.34 mm; 23.30 m/s); VI - stage No. 6 (0.25 mm; 23.30 m/s).

The inertial principle for capture of aerosols finds reflection in the Kishko instrument (1959). The apparatus is intended for determining not only microorganisms, but also dust and gaseous impurities in air (Fig. 27).

In designing an instrument for complex simultaneous investigation of the air, the author considered that investigation of the air for sanitation purposes can be considered fully accomplished if microorganisms, dust, and harmful gaseous impurities

in it are determined. The instrument uses the "slit" principle for microorganism sampling, with substantial improvements in the direction towards reduction of escape of microorganisms, portability, and simplicity of construction. In addition, the instrument makes it possible to carry out filtration of the air through liquid, paper, membrane, or gelatin filters. Depending upon the degree of assumed contamination of the air, for bacteriological research it is recommended to pass air at a rate of 10-25 l/min for 3-5 minutes. The apparatus consists of the air-sampling instrument itself, using a direct current of 12 V, and a power supply using alternating voltage of 110-127 and 220 V. During investigation the instrument is set on a folding metal stand, a convenient feature under field conditions.

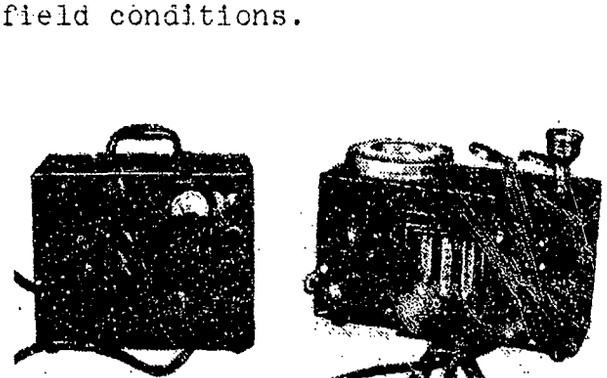

Fig. 27. External view of the Kishko general-purpose instrument.

Instruments which capture bacterial aerosols on a solid nutrient medium and which are based on the inertial principle have a number of advantages, including the following: seeding of air on the medium is carried out as the sample is taken; the instruments make it possible to extract large volumes of air over a prolonged period; parallel seedings give close and fully comparable research results; some instruments make it possible to carry out fractional determination of bacterial aerosols simultaneously with total quantities; industrial mass manufacture

of certain of the most effective types is fully realizable; the structure of the instruments is adequately simple and no special training is required to operate them.

Drawbacks of this method and instruments based on it include the following: with the exception of those designed on the impaction principle, inertial instruments do not capture highly dispersed fractions of the aerosols; viral aerosols are not captured; the majority of the instruments do not allow studying air under field conditions.

c) Deposition of Bacterial Aerosols by Electrostatic Forces

The possibility of using the principle of electroprecipitation to collect aerosol particles was outlined in Chapter II along with the essence of this method.

It is a known fact that in an electrical field particles, including those containing bacteria, are shifted toward the electrode whose charged sign is opposite to that of the particle. With this feature in mind various authors designed instruments which create an electrical field at the location of the nutrient medium. As the particles travel toward the electrode they encounter a dish with the nutrient medium. Microorganisms which reach the surface of the dish are deposited on it. Consequently, in essence the principle of electroprecipitation is a variety of deposition of the dispersed phase of aerosols; however, as a result of certain features it can be placed in a category by itself.

In 1888 V. I. Vartanov designed a precipitation bacterial trap. He used an ordinary beaker whose walls were coated with solid nutrient medium. Two electrodes were placed inside the bottle through two openings in the ends. The distance between

the electrodes was 10 cm. The tips of the electrodes were connected to the poles of an electrostatic generator. The vessel was opened in the area to be studied and, after filling with air, was closed once again. Then current from the electrostatic generator was applied to the electrodes and the microbodies inside the tank were deposited on the nutrient medium.

In 1934 Chizhevskiy used aeroionization in an electrical field for deposition of microorganisms out of the air and inside closed quarters, for purposes of sanitation and hygiene.

In 1935 N. S. Berov, P. A. Kouzov, and A. I. Semich compared the effectiveness of cleaning air contaminated by cement dust, which itself was preliminarily contaminated with a bacterial culture. The electrostatic filter on the deposition electrode contained 4-5 million viable molds and about 1 million living bacteria per gram of dust. However, during the development of the electroprecipitators it was soon found that they do not provide a high degree of capture of microorganisms out of air; therefore in all subsequently developed instruments the principle of electrostatic precipitation is combined with one of the methods of capturing bacteria on the surface of a solid nutrient medium.

Thus, in the Berry instrument (1941) electrostatic precipitation is combined with the "funnel" method; in instruments by Thompson, Rechmenskiy, Rooks, and Maisonnet it is combined with attachment by a strong jet of air; in the Uspenskiy and Lebedev instrument it is combined with the settling method, etc.

In his instrument Berry applied the "funnel" and electroprecipitation methods. A blower is used to pass air into a funnel which contains a box connected with one pole of a dc power source. The second electrode, charged with the opposite

sign, is located under a Petri dish. As is known, the majority of particles suspended in air have a negative charge. Aerosol particles pick up an additional charge by passing through the charged cell, after which they are attracted to the oppositely charged electrode.

According to Berry's data the use of the electrical field increases the instrument effectiveness by at least 2 times.

Luckiesh, Taylor, Holladey (1946) proposed an improvement to increase the effectiveness of the slit instrument. For this a voltage of 7000 V is applied to the supply and collecting portions of the instrument. The instrument consists of two vessels with removable covers. In one vessel the lower electrode is negative and the upper is positive, while in the other vessel the electrodes are reversed. The structure was developed on the assumption that both positively and negatively charged particles of bacterial aerosols are contained in air. The authors carried out a number of studies and obtained good results.

With atomization of a water suspension of E. coli in a room, about 10 times more bacteria were collected on a Petri dish on the positive electrode than on the negative. On the other hand, during collection of microflora of the air in closed quarters the greatest deposition (30% greater) was noted on the negative electrode. It is obvious that uncharged particles are also deposited on the electrodes under the action of gravity or inertial forces.

Luckiesh (1946) compared the collecting capability of an electrostatic precipitator and a slit instrument of his own design under open-air conditions. The average concentration obtained as the result of counting on the two dishes was 2-3% higher than the quantity determined by an instrument with a radial slit.

In an electroprecipitator designed by Rooks (1948) the electrodes are placed in a cup, after which the cup is filled with nutrient medium. A voltage of up to 14,000 V is created on the instrument. The effectiveness of the electroprecipitator grew in proportion to the increase in voltage on its electrodes. The instrument was tested with artificial bacterial aerosols. As a result it was established that the instrument collects about 95% of the microbe bodies contained in air.

The Maisonnet instrument (1956), which he called a "hydro-aeroscope," is based on electrical precipitation in combination with the impact effect of a stream of air and with filtration of air through a liquid. Passing through the "mesh" of the instrument, which contains 200 openings, the air successively passes through two electrical fields with voltage at the level of several thousand volts, where neutral particles pick up a charge and particles which are already carrying one are additionally charged; this is followed by deposition on the surface of the nutrient medium. In the first electrical field the upper electrode is charged to 10,000 V and the lower dielectric has a negative potential; in the second field the arrangement of the electrodes is the opposite. After passing through the two electrical fields the air is collected in a flagon filled with liquid, where microorganisms which were not trapped on the electrodes can also be retained.

N. D. Uspenskiy and K. P. Lebedev (1948) developed a simple method of electrostatic deposition of bacterial suspensions. A positively charged electrode is brought to a Petri dish. The depositing electrode is a metallic brush which is attached to the negative pole. The electrode can be installed at any height above a cup filled with nutrient medium. The difference in potentials on the terminals of the electric power source comprises 50,000 V; current strength is 2000-3000 mA. In sampling the

dish with the nutrient medium is installed on a settling table and then the electrical current is switched on. Upon termination of precipitation the dish is transferred to a thermostat.

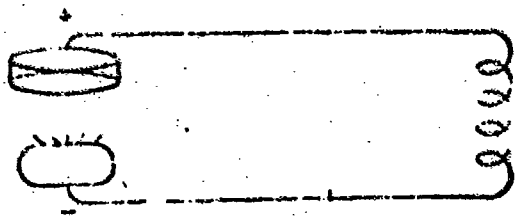

Fig. 28. Diagram of the Uspenskiy-Lebedev instrument.

Using their own method, the authors obtained extremely intensive deposition of bacterial bodies on the nutrient medium. This instrument is more effective than the Moulton apparatus (1943) and collects 10-100 times more bacterial particles than the Koch method. Figure 28 shows a diagram of the device.

Houwink and Rouvink (1957) developed two original types of electrostatic precipitators. The first of these is an instrument whose basic unit is a glass tube coated on the inside with a layer of nutrient agar. A high-voltage electrode is located in the center of a cylindrical collector. The layer of agar is grounded; a positive charge is applied to the central electrode. Deposition of microorganisms from air being aspirated through the tube is accomplished on the agar which lines the walls of the glass tube. In the second variant of the instrument a water-film collector is applied; this makes it possible to accomplish prolonged sampling of a bacterial aerosol. The water film is the negative electrode. A conductor located in the center of the tube is used as the positive high-voltage electrode. Microbe bodies are deposited in the liquid, which is then analyzed.

Soviet authors (A. Ye. Vershigora and V. V. Vlodavets) have proposed various electrostatic precipitators. In the

Vershigora instrument (1958) the principle of electrostatic precipitation is combined with the "slit" principle. Connection of a current 5 A strength and with a voltage of 12 kV increases the number of deposited bacteria by 8-10 times. V. V. Vlodavets (1959) used an electrostatic precipitator of his own design to carry out work on determination of the charges of microorganisms located in air.

There is substantial interest in the electrostatic aeroscope designed by Symon and Binek (1964). The instrument is a combination of an impactor and electrostatic precipitator. Particles pick up a charge in a corona discharge at the nozzle (negative electrode) and are attracted to a positive charge, where a dish with nutrient medium is located. In this instrument the cup is moved at the same time that it is rotated, so that the aerosol deposit has the form of a spiral, which in turn facilitates counting and identification of the bacterial flora.

Klobouk (cited by K. Spurný and coworkers, 1964) studied the possibility of using an electrostatic filter both in order to collect particles of an artificial bacterial aerosol containing Serratio marcescens under laboratory conditions and also to determine the degree of removal of bacterial particles from the air in hospitals. At a volume rate of air pumping equal to 300 m^3/h the effectiveness of deposition comprise 99.8% for particles containing Bacillus prodigiosus, while for bacteria in the air it was 97.32%. Thus, under hospital conditions the number of microbes ahead of the filter comprised 1454 (2456-992), while behind the filter there were only 15 (22-2).

Among the instruments designed on the principle of electrostatic precipitation we should take note also of the simplest electrostatic precipitator (O'Connell, Wiggin, Pike, 1960). Sampling is conducted by means of solid or hollow glass or plastic

cylinders which are given an electrostatic charge by rubbing them with a small piece of cloth, gauze, or short-haired fur. A cylinder thus charged is exposed in the investigated atmosphere for a certain period of time, after which it is rubbed several times on the surface of a solid nutrient medium. This method can be used to sample air both indoors and under field conditions. The designers of the instrument carried out a comparative evaluation of the charged glass cylinder and a liquid impinger and showed that the instrument which they proposed is not inferior in effectiveness to the liquid sampler. This instrument is not so suitable for quantitative as for qualitative investigation of the air.

On the basis of the material outlined above characterizing electrostatic precipitator instruments, it follows that electrostatic precipitators, possessing high capture properties, do not allow exact quantitative counting of microflora of the air; the apparatus tends to be complex in structure; it is not adaptable for operation under field conditions; it tends to be unsafe during operation and therefore training of the personnel is required.

d) Deposition of Bacterial Aerosols by the Thermal Precipitation Method

The method of thermal deposition is also a variety of methods of depositing a dispersed phase of bacterial aerosols, like electrostatic precipitation.

Proceeding on the basis of the principle of thermal precipitation as outlined in Chapter II, in 1952 Kethley, Gordon, and Orr designed a thermal precipitator for microbiological study of the air. This instrument operates as follows: air passes into a small space where the ceiling is heated by an electrical current to 80-100° and the floor is cooled by water. A cover glass, onto which the microorganisms will be precipitated,

is placed on the floor. Later the designers of the instrument replaced the cover glass with filter paper impregnated by heated agar. Upon termination of sampling the filter paper is placed on agar in a Petri dish. The instrument has certain drawbacks: rapid drying of the paper, low productivity in sampling air, and low velocity of the flow of investigated air.

Orr, Gordon, Kordecki (1958), in order to make maximum use of the advantages of the method of thermal deposition, developed a thermal precipitator for continuous sampling of an aerosol; in this device the aerosol particles are deposited on a moving strip or some other object. The instrument utilizes the properties of thermal precipitation: high productivity with respect to particle deposition and the possibility of direct investigation of the particles. In this instrument a paper tape or a film of plastic material is drawn slowly over a cooled plate, which is located below a heated plate. The air containing the aerosol particles is supplied to the plates and the particles are deposited on the strip (film). The purified air is drawn off along a channel located in the center of the heated plate. The basic unit in the instrument - the precipitator, through which the tape (film) is drawn - is placed in a housing located in the center of the instrument on its upper panel. As shown schematically in Fig. 29, the precipitator consists of cooled and heated elements which are located a small distance from one another.

A convex rectangular plate attached to the cooled element forms a chamber through which the cooling liquid (water) circulates. The heated element has a round shape, diameter 36.2 mm; its concave surface is located directly above the cooled element. The described instrument operates well at a temperature gradient of about 90.6°. The indicated gradient is achieved at moderate temperatures and with a distance between the heated and cooled surfaces of 0.5 mm. With a higher temperature the paper tape

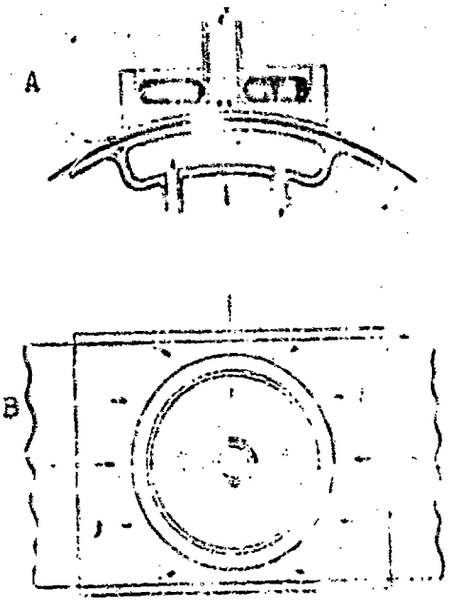

Fig. 29. Diagram of the deposition device in a thermal precipitator. A - side view; B - top view. 1 - electric heater; 2 - air outlet; 3 - sampling of air; 4 - strip; 5 - cooling.

is stretched and distorted, which disturbs conditions of normal deposition of the particles. The tape speed is 7.6-30.5 cm/h. At a rate of 1 l/min virtually all particles, located at approximately identical distances from one another, are deposited out of the air. The feed device consists of an electric motor with a time relay and with rollers which grip the edge of the strip without touching the precipitate.

Drawbacks of methods of aerosol sampling using thermal precipitation instruments include the dependence of their effectiveness on many variables: degree of dispersion of the investigated aerosol (all particles less than 20 μm are captured in a thermal precipitator - per Spurny et al, 1964), the rate of travel of particles under the action of thermophoretic forces, the rate at which air is pumped in the instrument, and so on.

3. Capture of Bacterial Aerosols by Filters

a) Aspiration of Air Through Solid Insoluble Filters

Capture of the dispersed phase of bacterial aerosols for microbiological analysis by means of filtration methods is carried out in the same way as sampling by these methods for physical analysis.

Deposition of the aerosol on fiber filters is caused mainly by the following effects: the inertial effect, catching effect, sedimentation, and diffusion of the particles. This circumstance led to substantial complexity in calculating the effectiveness of filters and as yet there is no single procedure for calculating effectiveness of deposition of an aerosol on filters.

Certain authors (Lond, 1957; Stairmandt, 1953) apply simple summation of the enumerated effects in calculating the effectiveness of filters, while others (Kuwobara, 1959) create a theory of filtration on the basis of viscous flow in a system of cylinders at small Reynolds numbers of the flow. In the opinion of N. A. Fuks (1961), the second method is the more promising.

The basic requirement which must be meet by the filters is effectiveness of deposition of aerosol particles; this is determined as the ratio of the number of aerosol particles deposited on the filter to the total number of particles in the investigated gaseous medium. Effectiveness of a filter is usually expressed as a percentage. Besides effectiveness, during determination of the concentration and degree of dispersion of the investigated aerosol it is necessary to know the selectivity of the filter, which will show just how the effectiveness of filtration will change as a function of particle size (3.2).

$$I = K \cdot \eta\,(\%), \qquad (3.2)$$

where I is the selectivity of the filter; η is effectiveness; and K is a coefficient which considers the change in degree of dispersion of the aerosol and equals the ratio of distribution of dispersion of particles deposited on the filter to the distribution of dispersion of particles in the air prior to filtration.

At present instruments in which various insoluble filtering materials are utilized for collecting microorganisms have found wide application.

As early as 1860 Pasteur indicated in his works that when air is passed through a cotton filter microorganisms will be retained in it, despite the fact that the size of the pores in the filter is substantially greater than the size of the microorganisms themselves. Pasteur expressed the opinion that particles suspended in the air collide repeatedly with fibers during passage of the air and are deposited on the outer layers of the filtering material.

Rubner (1907) applied filters of filter paper for investigation of the air. Subsequently such filters found wide application. Huberndick (1906), Sargent and Moller (1907), and others used this material. This method was applied in England to study contamination of atmospheric air by microorganisms.

The possibility of using paper filters as bacterial traps was studied in detail by Gonnel and Thomas (1925), who compared the capture capacity of paper filters with certain other methods of sampling air for bacterial seeding. The authors noted that the paper filter captured 10 times more microorganisms than sugar and sand filters.

Mironov, Krauze and Boyko (1940) carried out two series of bacterial studies of the air with paper filters and obtained good results. Their application of paper filters consisted in the following: filters were prepared from filter paper and inserted as a cone in an adapter 3 cm in diameter. The edge of the filter extended beyond the edge of the adapter and was clamped with a rubber ring. Air was passed through at a rate of 500-700 *l*/h. After passage of the air the filter was placed in a sterile jar with glass beads, to which 50 m*l* of sterile water was added. The obtained suspension represents material for determining bacterial seeding onto a nutrient medium.

Numerous studies established the high filtering capability of wool filters (Pasteur, 1860; Rousbery, 1947; V. A. Zubarev, 1954, and others).

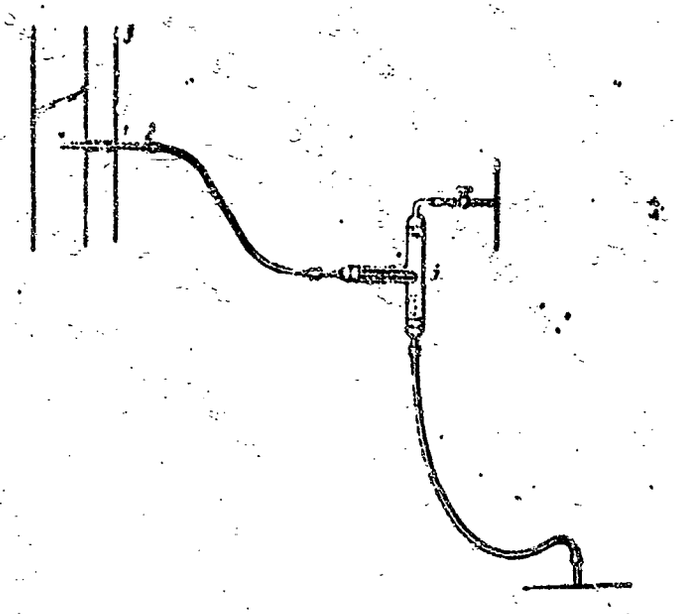

Fig. 30. The Pasteur nitrocellulose filter (see description in text).

The apparatus used by Pasteur for microscopic study of airborne dust was extremely simple (Fig. 30).

A tube 0.5 cm in diameter (4), containing a tampon of nitrocellulose wool (2), was exposed to outside air through a window frame (3). Air was drawn in by means of a water-jet pump (5), with the particles being deposited on the fibers of the wool. The volume of tested air was determined from the level of water displacement. After sampling the tampon was removed from the tube and dissolved in a mixture of ether and alcohol; the particles were deposited and the deposit was studied under the microscope.

Later glass fittings (extractors) or metallic tubes of various designs filled with cotton or glass wool were used for sampling. A specimen tube (extractor) is shown on Fig. 31.

A copper mesh containing the filter is placed inside the tube on a round projection. An aspirator is attached to one end of the tube. Microorganisms are deposited on the wool fibers and then, after washing in liquid, are seeded onto the nutrient medium.

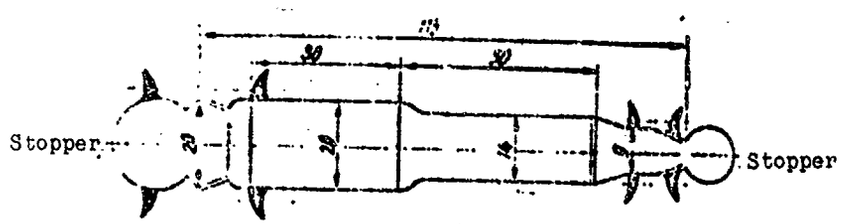

Fig. 31. Glass tube (extractor) (see description in text).

V. A. Zubarev (1954) designed a simple instrument (Fig. 32) in which the filtering material is hygroscopic wool placed in a metallic tube 100-150 mm long. The middle of the tube contains a mesh or simply 2-3 pins to hold the wool during aspiration of air.

During sampling of the air the tube with the wool plug is fitted onto the pump adapter. The manual pump has two rubber

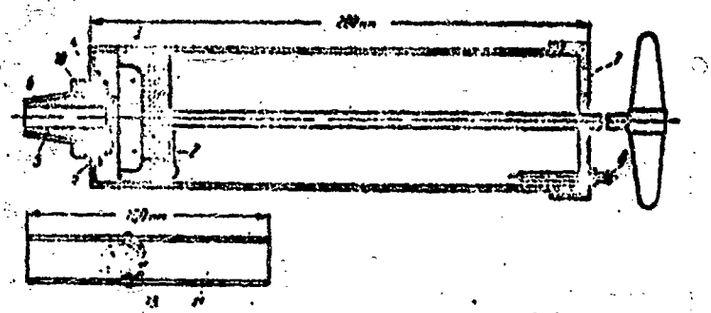

Fig. 32. Zubarev instrument.
1 - piston; 2 - valve; 3 - compression rings; 4 - valve; 5 - attachment for fitting the extractor; 6 - rubber tube; 7 - window in the pump cover; 8 - piston stop; 9 - rubber ring; 10 - adapter nut; 11 - extractor-tube; 12 - wool tampon; 13 - pins for retaining the tampon.

valves to ensure one-directional motion. The pump volume equals 1 l. According to data from the designer, at a rate of air motion through the extractor of 2.5-2.8 m/s there is virtually no penetration through the wool. After the air sample is taken the tampon is placed in a flask, 50-60 ml of sterile physiological solution is added, the mixture is shaken vigorously for 2-3 minutes, and then filtered through a membrane filter. For the sake of reliability a second washing of the wool can be carried out. Finally the liquid is seeded onto a nutrient medium. Tests of the instrument show that the wool tampon completely retains all microflora in the air; during washing with three successive batches of physiological solution the tampon gives up 96-98% of the retained microorganisms. Zubarev compared his instrument with the Shafir aspirator. In all cases, according to his data, the Shafir instrument recorded only 70-80% of the microorganisms as compared to readings from the tested instrument.

Studies of the effectiveness of wool filters (V. S. Kiktenko and coworkers) showed that the wool filter, with a layer thickness of 2 cm, actually retains all particles of a bacterial aerosol, but washing in three successive batches of physiological solution led to detection of no more than 5-10% of the retained microorganisms.

In 1956 I. M. Nikhinson and coworkers proposed a cartridge filter for bacterial investigation of the air; this unit is a cylinder 1.8 cm in diameter and 2.5 cm high. The bottom of the cylinder has 20 to 30 openings. A circle of 6 layers of gauze is placed on the grid of the inner surface of the cartridge and packed tightly by means of a second, bottomless cylinder. The authors carried out a number of experiments and established that this method captures substantially more microbes from the air than the D'yakonov instrument. Ordinary hygroscopic wool, glass wool, and filters of gelatin foam were used in 1958 by Ya. G. Kishko and V. I. Filimonov in designing their "pistol instrument" for studying bacterial seeding of air from aircraft. The instrument operates from the onboard power net of the aircraft, making it possible to pass at least 50-300 *l* of air through the filter. According to information from the authors, the instrument is easy to handle, portable, and applicable for virological investigations. In addition, the equipment can be used at negative air temperatures (Fig. 33).

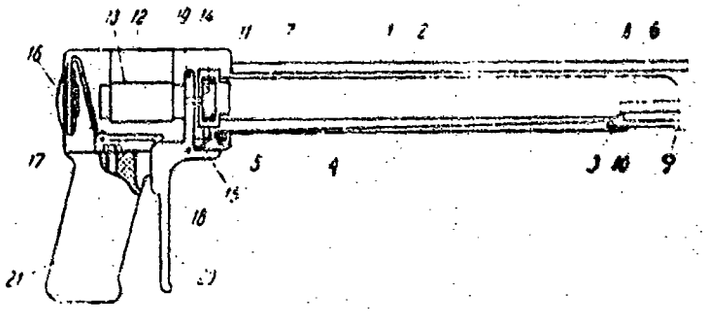

Fig. 33. Kishko pistol instrument (see description in text).

Duralium tube (1) is moved in the direction of slot (2) through a special opening in the ceiling of the aircraft cockpit by means of handle (21). The outer end of the tube has a rectangular window equipped with shutter (3). The shutter is opened and closed by means of cables (4) and a system of levers (5). The rectangular window has inlet aperture (6) for the inner air-guide tube (7) with socket (8) and clamps (9) for a cassette with the filter material (10). The instrument is operated by means of working parts (11, 12, 13, 14, 15, 16) and a system of levers (17, 18, 19, 20).

Considering the poor results of washing microorganisms out of wool fibers, V. S. Kiktenko, N. I. Kashanova, S. I. Kudryavtsev, and N. I. Pushchin (1961) proposed impregnants which would envelope the wool fibers and be easily washed off.

The instrument based on this collecting principle and subsequent desorption of the microbes is a glass tube of unique construction, filled with glass fibers (fiber thickness about 5 μm) or cotton wool impregnanted with a mixture of equal quantities of a 3% gelatin solution and vaseline oil. A specimen of the instrument is shown on Figs. 34 and 35.

The total length of the tube is 200 mm and the [greatest] diameter is 32 mm. The bacterial trap consists of three parts, each of which has its own function. The front part of the instrument (a) is in the form of a smooth tube 104 mm long and is intended to be filled with cotton or glass wool. The spherical portion of the instrument (b) serves as an additional reservoir for collecting drops of the impregnant in those cases when some of it leaks out of the wool when air is passed through the instrument. The end portion of the instrument (c) contains, in its expanded part, 2.5 g of cotton wool to retain random microorganisms which escape the main filter in the front part of the instrument. The additional wool filter is included to prevent

contamination of the pump, motor, and other parts of the entire installation by microorganisms. An aspirator is attached to the end of the instrument. The instrument should be made from glass no less than 1.5 mm thick.

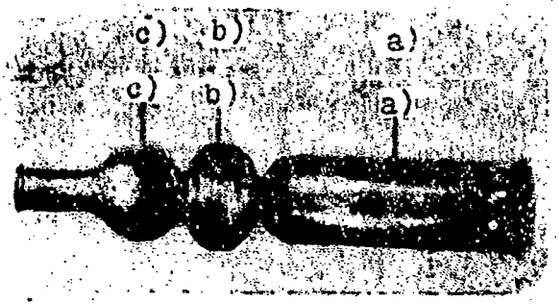

Fig. 34. External view of bacterial trap (see description in text).

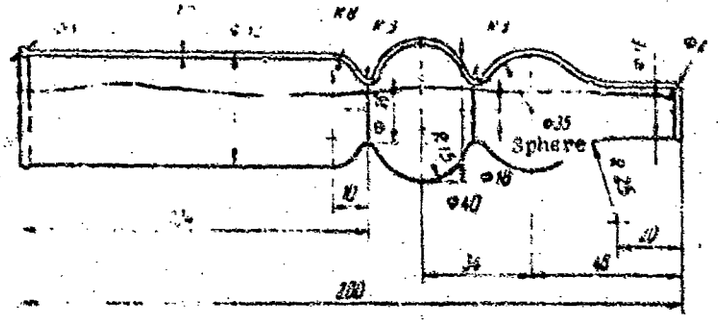

Fig. 35. Drawing of bacterial trap (see description in text).

The preliminary treatment of the instrument is as follows. Carefully washed and dried, the glass tube is filled with pure cotton or glass wool and the wool is washed out with distilled water. The quantity of glass wool is 10 g and that of cotton wool, 5 g. The wool must be packed as tightly as possible. The ends of the instrument are covered with wadded gauze plugs. After this the instrument is sterilized in an autoclave at 120° for 30 minutes or by dry heat in a Pasteur furnace at 160-170° for 40 minutes. The impregnant consists of equal quantities of sterile solutions of gelatin (3%) and vaseline oil, which are

mixed to the foamy state. 12 ml of the mixture is poured into one instrument, wetting approximately 3/4 of the wool filter. Impregnation of the wool is carried out under sterile conditions and immediately before sampling of the air. In addition to the mixture of gelatin and vaseline oil, such liquids as distilled water, physiological solution, 1% meat-peptone bouillon, a 1% solution of gelatin, a 15% solution of glycerin in physiological solution, and 1 and 3% solutions of meat peptone agar have been tested as wool impregnants. However, the capture properties are substantially increased when a mixture of vaseline oil and 3% gelatin solution is used to impregnate the wool.

Air sampling with the bacterial trap can be carried out by means of a dust pump, air blower, a manual pump, bellows, and other devices. Optimum conditions for operation of the filter are created when air is drawn in at a rate of 5-10 l/min. After sampling of the air the wool filter is removed under sterile conditions and washed in a flask with 50-100 ml of physiological solution or distilled water. Figure 36 shows fibers of glass wool before being washed in physiological solution, while Fig. 37 shows the fibers after washing.

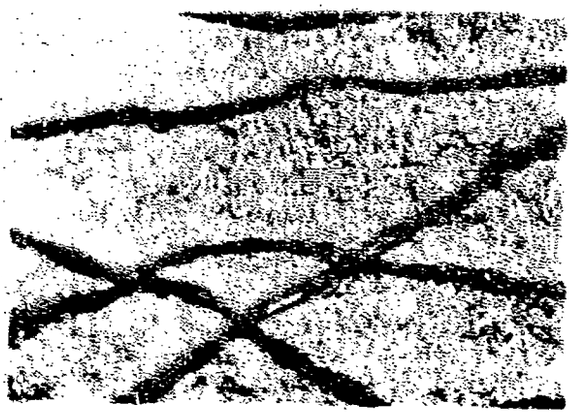

Fig. 36. Fibers of glass wool impregnated with a mixture of 3% gelatin solution and vaseline oil.

Fig. 37. Glass-wool fibers after the impregnant is washed off in physiological solution.

After the flask is shaken for 3-5 minutes the wool is carefully squeezed out and the washing liquid is used for subsequent bacterial research (seeding onto a medium, infection of animals, etc.) in order to accumulate the microorganisms retained by the wool filters. After squeezing the wool is placed on a solid nutrient medium and additionally cultivated. The simultaneous use of "washed" wool permits a significant increase in the quantity of positive research results. If filters of glass wool are used in the work, after it is ground up in a pestle with the addition of physiological solution or distilled water it is possible to carry out not only seeding but also infection of animals. During quantitative determination of microorganisms in air it is recommended that the following formula be used:

$$M = \frac{K \cdot O}{W \cdot B}, \qquad (3.3)$$

where M is the unknown quantity of microorganisms per 1 l of air; W is the quantity of investigated liquid (in milliliters), placed on one Petri dish with a solid nutrient solution; O is the total quantity of liquid (impregnant plus washing liquid in milliliters); B is the quantity of air (in liters) passed through the trap; K is the quantity of microorganism colonies grown on one Petri dish.

Evaluation of collecting effectiveness and the operational qualities of this bacterial trap was carried out in comparison with instruments of the Rechmenskiy, Vershigora, and Rudenko designs, a sorption instrument, soluble filters of gelatin foam (manufactured by the Vershigora recipe), and extractors filled with cotton and glass wool. The comparative study of collecting properties of the instruments was carried out under open-air conditions and also with use of an aerosol chamber with a volume of 0.5 m^3. In order to improve the collecting properties of the bacterial traps, there was created in the experimental chamber a liquid phase of bacterial aerosol of intestinal bacteria (Serratio marcescens, strain 20-10), hay bacillius (Bac. subtilis, strain 82-36) and also a polyvalent dysentery bacteriophage. Bacterial suspensions containing 0.5 billion microbe cells were prepared on tap water with addition of a 10% glycerin solution and 5% solution of saccharose; in addition, a gelatin-phosphate buffer with pH of 7 and the following recipe was used for the suspension of intestinal vacilli: gelatin 2g, Na_2HPO_4 4g, distilled water 1000 ml. The suspension containing spores of the hay bacillius was heated with shaking at 70-80° for 5-10 minutes before it was used.

Sampling was carried out by 4 instruments simultaneously, with 10 l of air being passed through each of them with regulation by flow meters. Sampling time was 1-3 minutes. The degree of dispersion of the basic mass of particles (86%) fell within the limits 3-15 μm.

During the period of sampling by the bacterial traps determination was made of the particulate concentration of the bacterial aerosol in the chamber by means of a photoelectronic counting unit. The results of these studies are shown in Table 12.

Table 12. Counted particulate concentration of aerosol in the chamber during sampling by the bacterial traps (average data from 20 series of studies).

Readings of photoelectronic particle counter (per l of air)							
Immediately after atomization	After 3 min	After 5 min	After 10 min	After 15 min	After 20 min	After 25 min	After 30 min
1,441,700	1,438,286	1,396,018	1,106,321	1,028,046	987,654	967,304	804,206

Calculation of aerosol concentration was carried out by the formula

$$a = \frac{N}{V} = \frac{d \cdot N}{W}, \qquad (3.4)$$

where N is the number of flashes counted; W is the volume of the aerosol; d is the constant for the given diaphragm opening with a certain optical system; and V is the volume of air in which the number of particles was counted.

As is evident from the data in Table 12, 10 minutes after atomization of the suspension the concentration of aerosol in the chamber was reduced by only 5% with respect to the initial concentration, determined immediately after atomization. Consequently, during the time of sampling no essential change in aerosol concentration was noted.

Results from study of the collecting properties of the bacterial traps of various designs with respect to the droplet phase of the bacterial aerosol containing intestinal bacilli and hay bacilli are presented in Table 13.

Thus, it follows from the data in Table 13 that the capture capability of wool filters impregnated with a mixture of 3% solutions of gelatin and vaseline oil is substantially higher than that for the Rechmerskiy, Verbigora, and Rudenko instruments, the D'yakonov sorption instrument, and for soluble filters of gelatin foam. The high capture capacity of bacterial traps with impregnants was also confirmed in experiments with capture of particles of dysentery polyvalent bacteriophage with atomization of the material in a chamber.

The insignificant quantity of microorganisms found in a filter of cotton and glass wool without impregnation is apparently explained by the death of cells of the bacilli, a fact confirmed

Table 13. Comparative average data of collecting of a number of microorganisms from chamber air by means of different instruments.

No.	Instruments and designers	Number of experiments	Average number of microorganisms trapped from 1 l of air	
			Intestinal bacilli	Hay bacilli
1	Bacterial trap of Kiktenko	25	2,270	3,280
2	Bacterial trap of Rechmenskiy	33	1,865	2,286
3	Bacterial trap of Rudenko	40	1,905	2,125
4	Bacterial trap of Vershigora	30	1,613	1,816
5	Bacterial trap of D'yakonov	15	1,305	1,862
6	Sorption instrument (glass impinger)	20	2,740	3,420
7	Soluble filters of gelatin foam (recipe of A. Ye. Vershigora)	24	1,046	2,648
8	Glass extractors with wool:			
	Dry			
	a) cotton wool	10	35	52
	b) glass wool	12	72	98
	Impregnated:			
	a) cotton wool + physiological solution	12	2,934	3,208

Table 13 (continued)

b) glass wool + physiological solution	13	1,320	1,865
c) glass wool + 1% solution of gelatin	27	12,425	15,262
d) glass wool + 3% gelatin solution	51	14,143	14,865
e) cotton wool + 3% gelatin solution	25	11,320	12,428
f) glass wool + .5% glycerin solution	8	330	1,986
g) cotton wool + meat-peptone bouillon	10	175	208
h) glass wool + 3% gelatin solution and vaseline oil (mixture)	50	35,036	38,208
i) cotton wool + 3% gelatin and vaseline oil solution (mixture)	50	22,534	24,081

Table 14. Coefficient of escape [of bacilli] in wool filters with respect to particles of an oil fog and a bacterial aerosol.

No.	Filtering material	Quantity of material	Escape factor (%)	
			Oil fog	Bacterial aersol
1	Glass wool	10 g	2.1	5.5
2	Cotton wool	5 g	0.08	1.05
3	Glass wool impregnated with a mixture of 3% solutions of gelatin and vaseline oil	10 g wool + 12 ml impregnant	8.1	0.35
4	Cotton wool with impregnant	5 g wool + 12 ml impregnant	0.1	0.025

by results of investigations with the hay bacillus. Attention should also be drawn to the poor results from washing microorganisms out of the wool fibers. The data presented in Table 13 found confirmation during studies of the retention capability of the instruments by the nephelometry methods with respect to particles of an oil mist and an aerosol of Bacillus prodigiosus. Particle size in the oil mist comprised 0.31 to 0.34 μm. The results of the study are presented in Table 14.

Membrane filters, used in the practice of research on water, were also tested for the study of microflora of the air. P. F. Milyavskaya recommends (1945) the following procedure for utilizing membrane filters to study bacterial contamination of the air. The membrane filters are boiled in water, dried, and placed in a Zeiss instrument or in special cassette filter holders (Figs. 38 and 39).

Fig. 38. Cassette filter holder for membrane filters, assembled.

Fig. 39. Cassette filter holder for membrane filters, disassembled.

Air is passed through the filter at a rate of 5 *l*/min. A rheometer is connected between the Zeiss instrument and the aspirator to calculate the rate of air intake. After filtration the membrane filter is placed with its mirror side on agar in a Petri dish and exposed in a thermostat for 48 hours.

Milyavskaya also recommends that the filter be washed in 15 ml of physiological solution. The washed filter and the liquid are seeded separately after filtration through several membranes. Calculation results are summarized. According to Milyavskaya's data (1946) and those from N. Korchak-Chepurkovskaya (1941), various bacterial cultures are grown successfully on membrane filters: staphylococci, streptococci, sporiform bacilli, etc.

An essential drawback of membrane filters is the fact that they do not allow passage of a large volume of air, owing to clogging of the pores by aerosol particles and increased filtering resistance. Ya. B. Reznik (1951) proposed using large-dimension filters to eliminate this drawback, since they would allow passing an enormously greater volume of air through the filter.

Since the first appearance of nitrocellulose membrane filters and up to the present the evaluation of their effectiveness has been accomplished. V. V. Vlodavets (1957) found that membrane filters trapped only 82.1% of the quantity of microorganisms determined by means of the Krotov instrument.

According to data from G. I. Sidorenko (1956), this percentage is much higher - 110.3%. Ye. V. Bragina (1957) established that membrane filters are inferior to the D'yakonov instrument in effectiveness. Krause (1948), using membrane filters to study bacterial seeding of the air, reported very high effectiveness for these filters - escape did not exceed 0.1% with respect to saprophytic microflora of the air.

The possibility of using membrane filters for studying bacterial contamination of air is also mentioned in works by a number of other foreign authors (Albrecht, 1957; Spurný, Jech, Sedlaček, Storch, 1964).

With direct cultivation of microorganisms directly on the membrane filters, somewhat understated results are obtained as compared with those obtained using aeroscopes. This is explained by the fact that microorganisms do not grow as well on membrane filters as on nutrient medium in a Petri dish (Albrecht, 1957) owing to the reduced penetration of nutrient substances into the body of the microbe through the filter. Besides this, the variety of particles deposited on the filter hamper growth of colonies. Dehydration of the microbes on the filter as air is passed through it also has an unfavorable effect. These drawbacks can be eliminated by dissolving the filter in methylene or ethylene glycol. In this way aerosol particles are converted into a hydrosol, which is cultivated on ordinary nutrient media (Mackala, Spurný, 1959).

Foreign investigators use membrane filters of the "Multipore" type (made of ash-free cellulose) with pore diameters of 0.8 to 5.8 μm (First, Silverman, 1953; Goetz, 1953).

Further development of the method of capturing particles of bacterial aerosol by the use of solid insoluble filters found a unique direction in the work by B. F. Sadovskiy, V. V. Vlodavts, Ye. Yu. Zuykovaya, L. I. Mats, and I. V. Petryanov (1963), who used a new type of filter, the "Mikrofil," to accelerate quantitative and qualitative determination of bacterial aerosols. The basis for the manufacture was the material FPP-5-8 (the Petryanov filter - chlorinated polyvinyl chloride). During short-term growth periods on the filter the particles of bacterial aerosol form microcolonies comparatively quickly; these can be revealed by staining with Pfeiffer fuchsine or with a 1% aqueous solution of methylene blue. The authors compared the possibility of accelerated determination of artificial bacterial aerosols using "mikrofil" filters and No. 4 membrane filters. Twenty-four hour cultures of Staph. alvus and Bac. mycoides were used as the experimental model of microorganisms.

When filters of the "mikrofil" type were used research time amounted to 5 hours and 20 minutes, as compared with 8 hours during application of the No. 4 membrane filter. The "mikrofil" filters possess substantial advantages (according to B. F. Sadovskiy and others, 1963) as compared with membrane filters and can be used in practical operations.

Decker, Buchman, Hall and Goddard (1963) evaluated the effectiveness of the following types of filters: electrostatic precipitators, air sprinklers, and scrubbers. The filters were tested with respect to bacterial particles of Bac. subtilis. The indicated authors consider that instruments containing filtering material of pressed glass, asbestos, and cellulose can be evaluated as moderately effective. At the same time the use of glass fibers, high-quality fiber paper, and asbestos with fiber diameter no greater than 5 μm ensures highly effective filtration (from 90 to 99% capture). Of the instruments in the air-washing class the most effective are those in which particles contact the wet surface and then are washed down. The effectiveness of these instruments varies from 20 to 90%. Electrostatic precipitators were also highly evaluated in the investigations by these authors.

The drawbacks of solid filters must include the reduction in effectiveness of deposition as the filter becomes clogged with dust and the consequent change in resistance to the flow during operation of the filter. Besides this, depending on the object of the investigation it is necessary to select a certain type of filter. At a certain weight concentration of the aerosol hygiene specialists use filters with minimum weight and minimum hygroscopicity; at a certain calculated concentration and degree of dispersion of the aerosol it is necessary that the material from which the filters are manufactured make it possible to study seeding of the particles with a microscope.

b. Aspiration of Air Through Solid Soluble Filters

Filters soluble in water were proposed for studying microorganisms suspended in air as long ago as the last century, and in recent years they have found wide development in various countries, primarily in the USSR.

In 1885 Foll used the aspiration method to filter air through ocean salt, which he then dissolved and studied the liquid for the presence of microorganisms. Somewhat later F. F. Lapchinskiy (1886), Miquel (1883), and others used sodium sulfate, sugar, acid sodium phosphate, magnesium sulfate, and other substances for the same purpose. These authors used simple research procedures. One or another soluble adsorbent was poured into a test tube and sterilized. A certain quantity of air was drawn through the prepared filter and then the adsorbent was dissolved and the solution seeded onto a solid nutrient medium.

However, as subsequent works showed, many of these substances raised the osmotic pressure in solution and as a result growth and development of microorganisms was inhibited. The growing colonies might be counted nine days after the experiment, instead of the 2-3 days during ordinary seeding of microflora from the air on meat-peptone agar.

In later years new types of soluble filters were developed which avoided this drawback - i.e., they did not cause a sharp increase in osmotic pressure. The American investigators Mitchell, Timmons, Dorris (1951) developed the technological manufacture of filters from gelatin foam which were soluble in water. The filters were prepared as follows (in brief outline): 4 ml of glycerin are dissolved in 100 ml of distilled water. The glycerin suspension is placed in a water bath at 52°, where 40 g of gelatin are added; the mixture is shakened and held for

20 minutes, after which the bottle with warm dissolved gelatin mixture is agitated energetically until air bubbles disappear and then mixed further with a special mixer; the obtained mixture is poured as a thin layer into boxes (12.7 × 12.7 × 3.8 cm) of parchment paper, which are then placed into a vacuum jar with potassium chloride. Air is removed from the jar with the pump until bubbles disappear completely and a layer of foam is formed; then the jar is disconnected from the pump and drying of the filter in the instrument is continued for 3 days. The dried gelatin foam becomes porous and has adequate tensile strength, where the porosity of the sponge is controlled by preparation of the gelatin layer in various thicknesses, drying the sponge in a dessicator for 1 hour at 120° and then sterilized by a gaseous mixture of ethylene oxide and carbon dioxide or at a temperature of 140° for 1.5 hours; filters of the required size are prepared from the dry gelatin foam by means of a special pattern.

In the USSR soluble filters of gelatin foam have been manufactured by A. Ye. Vershigora (1957) and by V. M. Shul'zhenko and A. A. Antonova (1959).

It should be noted that the procedures for preparing soluble filters of gelatin foam proposed by different authors have their own particular features.

Shul'zhenko and Antonova succeeded in obtaining filters with strictly determined pore openings, an extremely important factor in bacteriological study of the air. They developed and disseminated detailed instructions on the technique for manufacturing filters from gelatin foam.

For practical use the filters are placed in a filtering instrument which consists of a tube with an inlet opening and of a cassette for the filter (Fig. 40).

Fig. 40. Attachment for holding gelatin filters.

Upon termination of aspiration of the aerosol the filters are dissolved, placed in flasks with 100 ml of 0.85% solution of table salt and glass beads. The flask with the filter is heated on a water bath with periodic agitation for 30 minutes at 37°. The obtained emulsion is studied for the presence of bacterial or viral particles in it.

The effort to use gelatin filters to capture botulin toxin with subsequent centrifuging by setting up biological tests on white mice was undertaken by V. M. Khil'ko (1964). A droplet aerosol of botulin toxin with a concentration of 0.0027 mg/l and aerosol particle dispersion of 1 to 20 μm was created in an airtight chamber with a volume of 134 l. The aerosol was drawn from the chamber through a gelatin filter 3.5 cm in diameter and 0.3 cm thick located in a Zeiss apparatus. Aerosol particles which passed through the gelatin filter were captured in two successively located D'yakonov units.

Mitchell, Fulton, Ellingson (1954) carried out a detailed evaluation of the effectiveness of soluble filters of gelatin foam, comparing them with bacterial traps of the impinger, membrane-filter, and slit types. The designers of the filters showed that the procedure which they proposed for capturing bacterial aerosol particles is the most effective.

It was subsequently revealed that gelatin filters do possess certain drawbacks. Thus, Noller and Spendlove (1956) indicated that filters of gelatin foam are applicable mainly only for capturing the spore forms of microorganisms in air, since vegetative cells perish at the moment of aspiration because of the impact against the filter body. Another significant factor in the death of the microorganisms is the extreme dryness of the filter. These same authors indicated difficulty in dissolving the filter.

Filters of sodium glutamate (Kajiwara, Samori, 1954; Vanini, Campana, 1958) sodium alginate (Richards, 1955) and ammonium alginate (Hammond, 1958) have been proposed as filtering materials which are soluble in water and which do not cause a change in osmotic pressure, and are therefore suitable for studying bacterial seeding of the air. Sodium alginate is prepared from potassium alginate, insoluble in water, by wetting it in a normal solution of hydrochloric acid (to remove the potassium) with subsequent neutralization by sodium hydroxide. After washing with alcohol and drying a cottony mass (similar to wool) is obtained which is soluble in 10 volumes of water.

Filters for the glutamate are prepared and used as follows: crystals of sodium glutamate are ground to a particle size of 6-7 μm; 0.5 g of particles are placed in a glass cylinder (1.5 × × 1 cm) and sterilized at 160° for 30 minutes, whereupon the glutamate is dissolved; as it hardens pores 28 μm in size are formed in it. After sampling of the air the filter is dissolved in 10 ml of distilled water and seeded onto a dish containing nutrient medium.

V. V. Vlodavets (1959) considers that soluble filters of the alginate and glutamate of sodium, which do not contain nutrient substances for microorganisms, are very convenient. After air is sampled there is no need to carry out seeding immediately, since the microorganisms do not multiply on the filters. In

filters of gelatin foam favorable conditions for multiplication of the microbes can occur because of moisture accumulating during filtration of the air.

In 1963 Ye. P. Sinel'nikova proposed using liquid and foam filters, widely applied because of their high effectiveness to capture chemical aerosols (A. N. Fuks, 1955), for capturing bacterial aerosols. The best foaming capacity is found in colloidal solutions - saponin, peptone, gelatin, albumin, pectin, and casein with addition of surfactants - alcohols, organic acids and their salts, etc. Foams absorb aerosols due to sedimentation and diffusion. Sinel'nikova tested foams obtained from a 1% solution of sodium alginate, 1% peptone, whole and half-diluted meat-peptone bouillon, 30% glycerin solution on meat-peptone bouillon, and distilled water. A variety of the Ramey method was used to study the bacteria-retaining properties of the foams; the water in this method was replaced by the foams. Instead of a cylinder a glass funnel with a No. 2 porous filter was used. Staph. albus and E. coli were used as models of the experimental bacterial aerosol. The most sharply expressed absorption capacity was found in foam filters obtained on meat-peptone bouillon and a 30% glycerin solution on meat-peptone bouillon and distilled water, taken in a volume of 0.5 ml. The escape factor through the indicated filters varied in limits from 0 to 0.49% (average value 0.38%).

In conclusion it should be noted that filters of materials which are soluble in water or in physiological solution possess a number of positive qualities: higher flow-rate capacity; simplicity and convenience in handling; prolonged retention of microorganisms (spores) in the filter fibers, owing to the absence of nutrient substances for their multiplication, and accumulation of moisture sufficient for them to retain viability; the possibility of carrying out virological studies; and the possibility of using them at negative temperatures.

Drawbacks include failure to trap highly dispersed particles of bacterial aerosols, relative complexity of filter manufacture, and the death of a part of the vegetative forms of microorganisms at the moment of impact on the filter fiber.

c) Aspiration of Air Through Liquid Filters

In 1860 Pasteur first used liquid nutrient media for the deposition of bacteria. Subsequently new methods of collecting microorganisms with liquid filters were proposed.

Work was carried out in this direction by Miquel (1889), Emmerich (1883), Straus and Wurtz (1888), Rettger (1910), P. P. D'yakonov (1925), A. Ye. Vershigora (1956), S. S. Rechmenskiy (1951), V. S. Kiktenko (1956), and others.

Miquel passed fixed volumes of air through sterile meat bouillon and other nutrient media poured into vessels in amounts of 50 or 100 ml. He carried out wide-scale investigations of bacterial contamination of air in various types of terrain at different times of the year, depending on the effect of weather, height above sea level, and other factors. The research method proposed by Miquel entered rapidly into the practice of sanitation research on the air after the year 1883.

Bacterial traps proposed in following years by various authors are distinguished mainly by different structures of the vessel in which the liquid is placed, different sizes and shapes of the outlet opening of the tube through which the air is pumped, and by the presence of glass beads or openings in a plate in the vessel, intended to break up bubbles of air, along with other particular features.

When air is passed through a liquid in the form of fine bubbles various combinations of inertial, sedimentation, and diffusion deposition are observed.

One of the simplest structures is the D'yakonov instrument (1925), which is widely applied even at present.

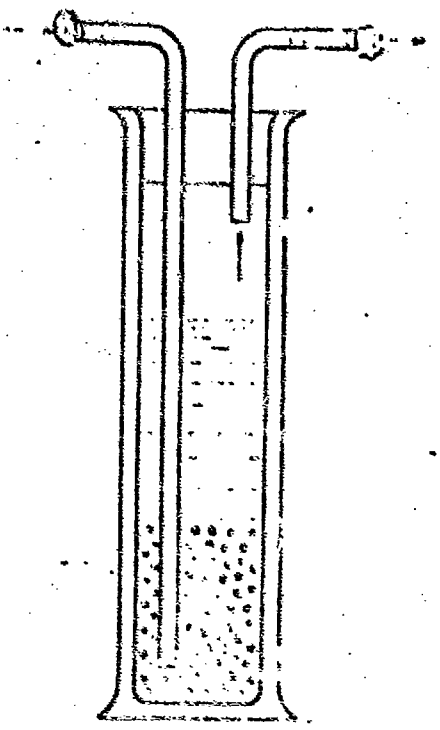

Fig. 41. D'yakonov instrument.

The instrument (Fig. 41) consists of a cylinder filled with glass beads and liquid (water, physiological solution, etc.) in a quantity of 30-50 ml. The cylinder is closed with a stopper through which two tubes pass: input and output, ending immediately below the plug. The outer openings of the tubes are closed with cotton stoppers. A monitoring plug can be placed in the horizontal elbow of the output tube. The assembled instrument is sterilized. Before use the cotton plugs are removed.

Air is pumped in the direction indicated in Fig. 41. After sampling the contents of the cylinder are carefully decanted and seeding of the liquid is carried out.

A. A. Adamova and M. A. Krivetskaya (1939) consider that the D'yakonov method is sufficiently accurate for study of bacterial contamination of the air. It is a fact that almost all investigators concerned with bacterial seeding of the air compared newly developed methods with the D'yakonov instrument. Vlodavets indicates that the D'yakonov instrument traps 59.9% of the quantity of microorganisms determined by means of the Krotov instrument (1957). S. I. Nasledysheva and A. P. Miroshnikova (1940) established an escape factor of bacterial aerosol in the D'yakonov instrument amounting to 50%. P. F. Milyavskaya (1945) improved the D'yakonov instrument. She proposed that the air stream be broken up into fine bubbles not only by beads by also by extremely fine openings (diameter 0.2 mm) in a quantity of 15-20 in the blind end of the intake tube. Rettger (1910) made approximately the same change in the D'yakonov instrument. The end of the intake tube in his instrument is closed over and about 20 openings are made in it. The instrument is filled with 50 ml of solution and sterilized in this form. N. K. Krontovskaya and F. G. Krotkov and coworkers (1946) detected rickettsia in indoor air by means of the D'yakonov instrument.

Wheeler (1941) used a Bunsen ring with side openings in his studies. A flask was filled with bouillon and glass beads were immersed in it. A glass tube enters through a rubber stopper; one end of the tube is closed and has a few fine openings. Air passing through these openings is broken up and overcomes the resistance of the beads immersed in the solution. This favors the retention of fine bacterial particles. During comparison with the Wells centrifuge it was found that the D'yakonov-Wheeler instrument traps 8 times more bacteria from the air.

An essential drawback of the majority of these instruments representing modifications of the D'yakonov bacterial trap is the fact that air passes through the liquid in the form of fairly large bubbles and only those bacteria on the surface of the bubbles are subjected to absorption.

For this purpose A. I. Shafir (1951) used a Drexel bottle; Lemon (1943) used a Folin tube from the van Slyke instrument for determining urease; Cvjetanovis (1955) used a 70 ml test tube; Fomin (1957) used the compressor from a Liebig cooler; V. S. Kiktenko and coworkers (1956) used a V-shaped tube with a large quantity of glass beads, etc.

The instrument designed by Kiktenko and coworkers (1956) is a V-shaped tube 25 cm in length and 1.5 cm in diameter. The tube is connected by a rubber adapter to a bottle tipped bottom up, which contains a tubule. The capacity of the bottle and the tubule is 250 ml; the height is 14 cm, diameter 6.5 cm, and the tubule opening, 1.5 cm. In assembled form the instrument is a system of communicating vessels which differ in diameter and volume.

A diagram of the device is shown on Fig. 42.

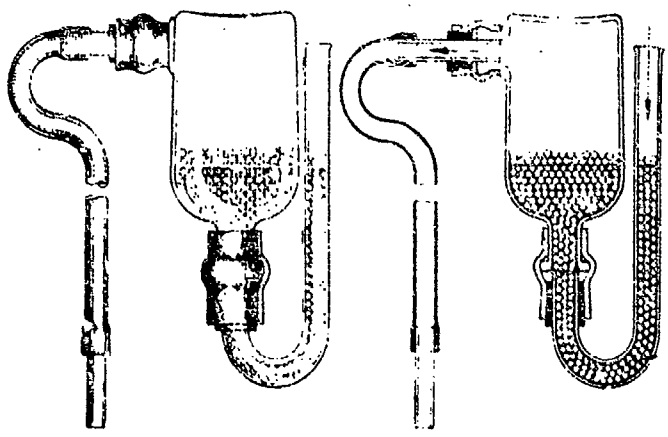

Fig. 42. Kiktenko device.

The inner portion of the tube and part of the vessel are filled with glass beads made from neutral glass; the grain size is 6 mm and the total surface reaches 750 cm^2. Forty ml of physiological solution or bouillon (peptone water) are poured into the instrument. A rubber tube 30-40 cm long is attached to the tubule of the instrument.

A continuous pump or human breath can be used as the means to ensure motion of the air during operation of the unit.

When the unit is activated by breathing the aspiration of air is not continuous and as a result there is periodic aspiration of air through the liquid. When the air is drawn by a pump the liquid is displaced from the tube into the bottle, and thus the beads wet by the liquid are uncovered and collection of bacterial aerosol occurs on them. When breathing halts, the liquid is returned from the bottle into the V-shaped tube and washes off the microorganisms adsorbed on the beads.

Using this instrument, Kiktenko collected 2.8 times more microorganisms of coliform bacteria than by using the D'yakonov instrument.

In 1958 A. Ye. Vershigora proposed a bubbling bacteria trap, representing a glass cylinder 2.5 × 35 cm in size sealed shut at both ends. A glass plate is fixed in the cylinder 6 cm from the bottom; it contains 30 openings 1 mm in diameter. A second plate is placed 6 cm above the first.

An air intake tube 0.5 cm in diameter is located parallel to the outer wall of the cylinder and communicates with its cavity 2 cm from the bottom end. A tube 0.5 cm in diameter, ending 2 mm from the bottom of the center of a contracting opening 2 mm in diameter is detached to the inner opening of the intake tube. Ten cm from the upper plate the housing of the cylinder is expanded (diameter of expanded section - 5 cm).

The air exhaust tube is located at the upper end of the cylinder.

Twenty ml of physiological solution or of a 1:9 mixture of meat-peptone bouillon and physiological solution is placed in the cylinder (Fig. 43).

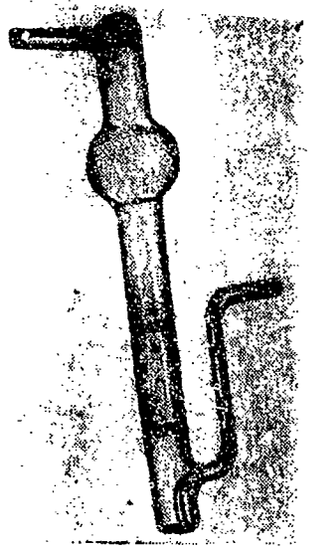

Fig. 43. Vershigora instrument.

The effectiveness of microbe detection in the bubbling bacterial trap in the case of a coarsely dispersed aerosol corresponds approximately to the effectiveness of the D'yakonov instrument. According to data by the author, the instrument is more convenient to operate than the D'yakonov bacterial trap.

In 1961 Glosclaude, Hermier proposed for the first time that the aerosol be passed not through water, but through dry beads with subsequent addition of 10 ml of water. The filter itself consists of a copper tube 101.6 mm long. A porous bronze disc 8 mm thick is located inside it. Glass spheres 50-100 μm in diameter are placed on this disc. Air is drawn through the filter at a rate of 7.9 to 8.5 m^3 per hour. After sampling the beads are agitated in the flask with 10 ml of diluent and the condensation tube is also washed out with the solution. Seeding is carried out from both liquids onto solid nutrient medium. The growing microorganisms are counted and identified.

Glass liquid bacterial traps known under the general name of impingers are presently used very widely in both laboratory

and field conditions in the USA and in England. All impingers operate on the principle of drawing air through an intake tube from a limited capillary opening. This leads to the formation of extremely fine bubbles and accelerates passage of the particles up to a velocity sufficient to cause deposition in the liquid. Simultaneously the capillary serves as a limiting opening, creating a gas-flow velocity which is virtually independent of the differential pressure between 0.5 and 1 atm; this eliminates the need to control the flow externally. Different flow velocities can be obtained by appropriate change in the diameter (passage) and length of the capillary. A brief description is given below of liquid samplers most widely used outside the Soviet Union.

The first liquid impinger was described in 1922 by Grenburg and Smith; it was intended for evaluation of the dust content of the atmosphere. Rousbery (1947) and Henderson (1952) give a procedure for using instruments of this type in their works.

The Rousbery-Henderson capillary filter is a small bottle equipped with a wide intake tube, with a short capillary soldered to the inner end of the tube (Fig. 44).

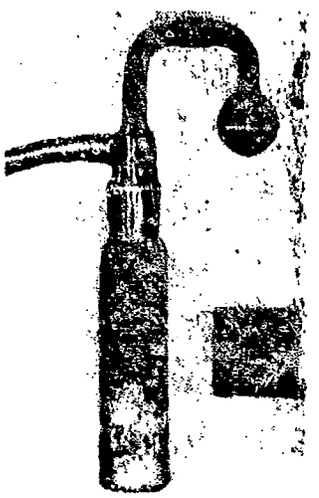

Fig. 44. Rousbery-Henderson sampler.

The capillary is immersed in the liquid no more than 5 mm, at a distance of 4 mm from the bottom of the bottle. Air is passed through it at a rate of 2-3 up to 20 l/min. The filter was tested out in a number of investigations and showed high effectiveness with respect to capture of highly dispersed bacterial aerosols. To avoid losses of aerosol particles

larger than 8 μm, which are deposited in the column of the intake tube, May and Druett (1953) proposed installing a prefilter in the instrument.

Tylor and Shipe (1959) described the Porton impinger and a standard impinger identical to it (Fig. 45).

Fig. 45. Standard impinger.

The instrument is intended for isokinetic sampling of aerosols. The aerosol sample to be analyzed enters the impinger along a bent tube, which terminates in a nozzle. The nozzle is a short capillary tube with a critical opening. The distance between the nozzle and the bottom of the flask in the standard impinger is 4 mm. At a vacuum of 0.5 atm and more the critical opening of the nozzle ensures a constant sampling rate. The optimum volume velocity during operation of the instrument is 11 ℓ/min with a nozzle diameter of 1.1 mm. In this case there is virtually no death of spores during deposition; the loss of vegetative cells is approximately 25%.

In order to increase the effectiveness of sampling with aerosols consisting of vegetative cells various modifications of the standard impinger have been designed. The basic change deals with an increase in the distance between the nozzle and the bottom of the flask or a change in the shape of the nozzle (along the lines of a Venturi tube or a truncated cone - the subcritical impinger).

An all-glass impinger (all-glass impinger, "Agi") is a highly improved standardized variety of the Porton impinger or the standard impinger (Fig. 46).

Fig. 46. All-glass impinger.

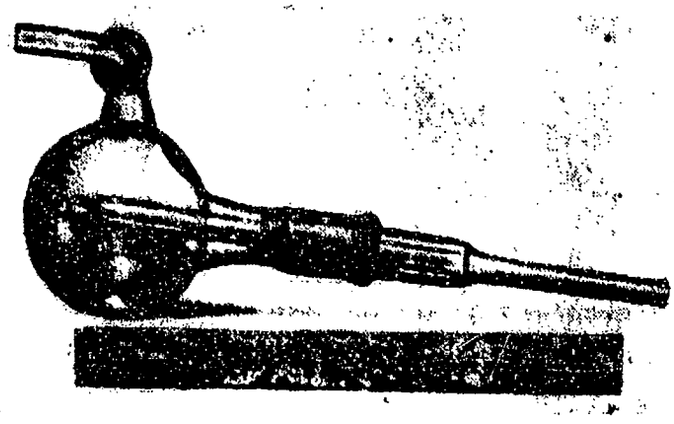

Fig. 47. Canadian impinger.

Canadian impinger. This sampler (Fig. 47) was proposed by researchers at the Saffield Experimental Station and was based on the principle of reducing the death of bacteria by bringing in the stream of air along a tangent to the liquid.

Shipe, and Tylor (1959) carried out a detailed study of the capture properties and operational qualities of impingers of different designs. A comparative evaluation was carried out with wool samplers which, according to data from the authors, capture a greater number of aerosol particles from the air than do other instruments. The suspension of bacterial aerosols was created in a special chamber using cultures of such microorganisms as Bac. subtilis and Serratio marcescens. On the basis of data obtained by these authors it is obvious that the "Agi"

instrument mainly collected just as many spores of Bac. subtilis as a supposedly better "absolute" sampler - the wool-type collector. The "Agi" instrument demonstrated the greatest effectiveness with respect to highly dispersed fractions of a uniform bacterial aerosol (particle size within limits of 3 μm). However, with nonuniform droplets other impingers proved to be more effective than the "Agi" instrument.

Although liquid impingers differ from one another in structural details, operating conditions, and effectiveness, the following particular features are characteristic for all of them: compactness and simplicity of construction, the possibility of seeding the collected liquid on different nutrient media, the possibility of using a series of checks to cover a broad range of concentrations of microorganisms, investigation of viral aerosols, and exceptionally high effectiveness in collecting particles larger than 0.5 μm in idameter, along with a constant rate of air passage.

A drawback of liquid impingers which is of critical importance in certain studies is the fact that they do not permit analysis of the bacterial sample directly in the instrument. This drawback is lacking in slit instruments and cascade impactors, within which separation of bacterial particles occurs directly on the solid nutrient medium.

4. Instruments Based on the Principle of Deposition of Bacterial Aerosols by Vapor or Atomized Liquid

Instruments constructed on the principle of deposition of bacterial aerosols by vapor or by atomized liquid are based on disturbance of the equilibrium of the system of bacterial aerosols which can be drawn in by vapor or by atomized liquid. These include the following instruments: Le Gujon-Grooten

(1902-1906), Elliot (1941), Moulton (1943), Rechmenskiy (1951) and Rudenko (1956).

In 1943 Moulton proposed an instrument based on the principle of capturing particles of an investigated aerosol by extremely fine drops of a liquid medium dispersed by a jet of pumped air. In the Moulton instrument the air enters a lower opening through a bent side tube, within which there is a siphon. Extremely fine droplets of liquid which are formed in this tube are captured in a pear-shaped receiver which contains bouillon. Air passes into the opening of the instrument at a rate of 300 cm^3 per minute. The bouillon of the middle chamber, enriched with microorganisms, is mixed with bouillon in the bent tube and seeded onto selective media in volumes of 0.1, 0.5, and 1 ml.

The Moulton instrument can be used to detect up to 80% of bacteria suspended in air. However, the instrument is complex in design and not easy to operate. Besides this, the instrument is not designed for passage of large volumes of air. Somewhat later S. S. Rechmenskiy (1951) used the idea forming the basis of the Moulton apparatus and proposed an instrument of his own. The siphon receiver of this instrument (Fig. 48) is a glass cylinder (1), whose inlet opening (2) enters a funnel which terminates in a capillary (3), directed into the cylinder. A second capillary enters the capillary of the funnel tube at a right angle (4); the second capillary rises first to a special reservoir (5) located below the cylinder, into which the collecting liquid is poured (physiological solution, bouillon, etc.).

Thus, the glass tubes form a pulverizer. On the opposite and somewhat contracted end of the cylinder there is a rubber tube (6) which joins the siphon receiver with the air aspirator and a rheometer. The upper wall of the cylinder contains an opening to which a rubber tube with a glass spatula is attached (7 and 8).

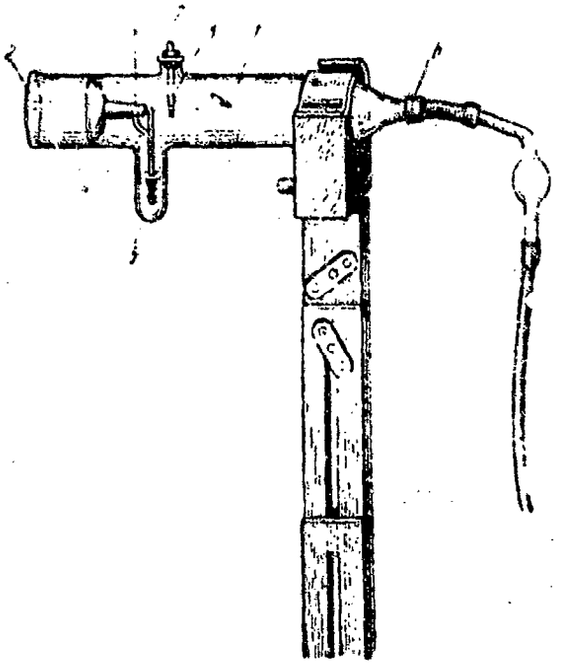

Fig. 48. Model of the Rechmenskiy instrument (see description in text).

Preparation of the instrument for operation and its operating principle are as follows. Some 3-3.5 ml of liquid is poured in through the upper opening (7) into reservoir (5) and it is closed with a rubber stopper with a glass spatula. The intake and outlet openings of the cylinder are closed with sterile wool plugs and paper caps. In this form the instrument can be sterilized in an autoclave.

During operation the instrument is attached to the aspirator, with an ordinary exhaust pump being used as the latter. As the jet of air is drawn through the horizontal capillary the capturing liquid from the reservoir is raised up along the vertical capillary to the horizontal one. Here it encounters the air stream and is broken up. An aerosol of nutrient medium is formed and absorbed on the bacterial particles, increasing their mass and thus improving deposition. This is facilitated by the spatula (8), which is agitated against the stream of liquid. The liquid runs down over the walls into the reservoir and the previous cycle is repeated. This makes it possible to concentrate the

microflora contained in large volumes of air (up to 100-150 ℓ) in a small volume of liquid. Upon termination of aspiration of air through opening (7) a sterile pipette is used to draw the liquid out onto ordinary nutrient media, depending upon the purposes of the investigation.

According to literature data, the Rechmenskiy instrument possesses good trapping capability as compared with other devices.

Thus, Ya. G. Kishko (1959) considers that the Rechmenskiy device possesses high capture capability with respect to all phases of bacterial aerosols. According to data from G. I. Sidorenko (1956), the Rechmenskiy siphon bacteria trap captures 42% more microorganisms from the air than the Krotov instrument, while observations by Vlodavets (1957) show a 39% greater capture.

The Rechmenskiy instrument has a number of drawbacks. Thus, the aerosol can be drawn in only when a powerful fan is available. Because of complexity in manufacture it is difficult to standardize the instrument, which leds to difficulties in obtaining uniform research results when using different instruments. The device is inconvenient for studying bacterial seeding of the air at low (negative) temperatures, since the liquid in the capillaries freezes rapidly (V. V. Vlodavets, 1958).

In 1956 N. M. Rudenko also proposed a bacterial trap based on the pulverization principle (Fig. 49).

The effectiveness of this instrument was studied by V. S. Kiktenko and coworkers, who found that use of the Rudenko type instrument led to capture of double the number of microorganisms from air as the siphon bacterial trap by Rechmenskiy.

5. Methods of Virological Investigation of Air

Instruments constructed on principles of filtration of aerosols through a liquid or deposition of aerosols by vapor or atomized liquid can be, under certain conditions, used for virological investigation of the air.

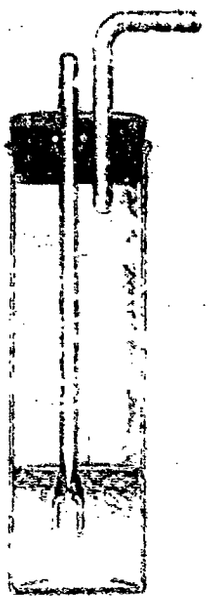

Fig. 49. Rudenko instrument.

In the opinion of G. I. Karpukhin (1962), particles of an aerosol which contain viral bodies can be determined by means of the instruments designed by D'yakonov, Kiktenko, Rechmenskiy, Rudenko, and others. A. Ye. Vershigora, Ya. G. Kishko, and V. V. Vlodavets consider that the Rechmenskiy siphon bacterial trap is fully suitable for virological research. Thus, Kh. L. Galikeyev (1956) detected the influenza virus in the air of an experimental chamber with the Rechmenskiy unit. Albrecht (1957) successfully used liquid impingers and membrane filters with subsequent special cultivation of hydrosols for the investigation of viral aerosols. Thorn and Burrows (1960) showed experimentally that samples of an aerosol of the hoof-and-mouth disease and certain other viruses can, at least quantitatively, be taken by standard methods of sampling bacterial aerosols. The authors obtained positive results in tests of standard impingers of the Porton type, as well as various modifications of it. The impingers were filled with an absorbent - a buffered phosphate or salt of a buffered phosphate. In addition, solid insoluble filters were tested: membrane filters and filter paper. After passage

of air through the instruments, subsequent investigations were carried out by ordinary virological methods (setting up serological reactions, infection of animals and embryos, tissue culture, etc.).

Together with I. A. Yurikas (cited by Karpukhin, 1962), G. I. Karpukhin made an effort to separate the influenza virus from air by means of the Shafir centrifuge. The nutrient medium in the apparatus was replaced with a suspension of sheep erythrocytes (12-15 ml). The influenza virus was atomized into an experimental chamber. Two-hundred fifty - two hundred l of air were passed through the apparatus. After sampling the virus was removed from the erythrocytes by elution. The wash liquid was investigated serologically. Positive results were noted in all 11 experiments. After elution the virus was found in the remaining water in dilutions of 10^{-2}-10^{-}[omitted].

In 1959 S. Ya. Gaydamovich, V. V. Vlodavets and V. R. Obukhova developed a procedure for capturing the influenza virus by means of the D'yakonov apparatus and soluble filters of gelatin foam.

Artenstein, Cadigan (1964) used the "Agi" type all-glass impinger with a tissue culture in a liquid medium to capture adeno viruses and the influenza virus from air.

Conclusion

As this survey indicates, the quantity of methods used in sanitation bacteriological investigation of the air is very great and continues to increase with every passing year. However, existing procedures and the bacterial traps based on them are still far from perfection - a fact indicated by their great variety. Up to now there is no single widely accepted and easily

accessible procedure which satisfies all of the requirements imposed on methods of capturing bacterial aerosols.

Considering the particular features inherent to bacterial and viral aerosol systems and also the knowledge of the physio-chemical laws governing the behavior of aerosols in general, at present there is every basis for a clear formulation of the requirements which must be met by instruments intended for capturing microorganisms from the air.

These requirements can be reduced to the following positions:

1. Separation of microorganisms from the air and their concentration in a liquid or solid medium should be completely possible.

2. The instrument must make it possible to select the greatest possible volume of air in a sample, since the concentration of microbe cells in the investigated air may be very small and their distribution can be extremely irregular.

3. Microorganisms captured by the instrument should not lose viability and their concentration in the material should permit their further study (seeding on various media, infection of animals, etc.).

4. The instruments should capture all fractions of bacterial and viral aerosols: large-nucleus, small-nucleus, and bacterial dust.

5. The instrument must, as far as possible, permit determining both the particulate concentration and the state of dispersion of the aerosol.

6. The conglomerates into which airborne microorganisms may be united should be broken down into individual bacterial or viral particles.

7. The instrument must be simple, convenient, and economical.

Sampling should not take a long time and should be accomplished both indoors and outdoors.

Besides this, one must consider also that during quantitative and qualitative analysis of a sample of a biological aerosol the following difficulties may be encountered: certain types of microorganisms do not grow or grow poorly on ordinary nutrient media (for example, viruses and various types of rickettsia) and therefore they cannot be detected; many types of bacteria and fungi grow poorly on solid nutrient media and require special nutrient substances or special growing conditions; microbes which are injured during sampling may not multiply in the nutrient medium; different types of microorganisms present in a single sample may prevent each other's growth or terminate it altogether; dust particles of certain chemical compositions and other forms of aerosols which are sampled together with the microorganisms may inhibit the growth of certain types of microbes.

During practical study of the concentration of microorganisms it is necessary to consider all of these factors and to select the appropriate research methods.

Consequently, in working with bacterial aerosols the use of one or another research method is determined primarily by the stated problems and goals of the investigation.

The requirements imposed on bacterial traps are met most completely by methods based on filtration of aerosols through solid insoluble and soluble filters (wool filters with easily removed impregnants, filters of gelatin foam with precisely

calibrated pores, filters of sodium alginate and sodium glutamate, etc.). Filters of glass and cotton wool retain all fractions of bacterial and viral aerosols and permit the passage of large volumes of air; in the case of wool filters with impregnants they retain the viability of microorganisms for a prolonged period and are simple and convenient to handle.

Methods based on adsorption of aerosols by a liquid are also adequately sensitive (sorption instrument, standard impinger, etc.). However, with small concentrations of microorganisms in the air the need to dilute the liquid leads to very high recalculation coefficients, reflected in the accuracy of the obtained quantitative results.

It should be noted that the structural features of bacterial traps should correspond completely with the structure of the bacterial aeroplankton.

During selection of the method it is necessary to know at least approximately the microorganisms which must be dealt with, the size of the particles that must be captured, etc.

The greater the variety in the type, size, and degree of agregation of microorganisms in the air, the more difficult it is to select a method for determining their concentration in the air. Thus, aerial bacterial suspensions - large drops of dust - should be captured by the method of sedimentation and the impact effect of an air stream. For detection of highly dispersed bacterial aerosols it is advisable to use methods based on the principle of adsorption, electrical or thermal precipitation, or siphoning of the air. Here it is necessary to consider whether the investigation is to be of bacterial contamination of air inside a room or under outdoor conditions, where aerodynamic effects are obtained.

In the majority of cases bacterial traps are designed for evaluation of the bacterial seeding of indoor air; therefore the corresponding apparatus has been developed with consideration of the relatively fixed or slightly moving air and the effectiveness of aerosol particle capture has usually not been given proper attention. During sampling of atmospheric air the aerodynamic effects play a basic role in the process of aerosol particle deposition. On this basis the use of various surface traps (object glasses with a sticky surface or Petri dishes with nutrient medium), despite their availability and simplicity, may give only approximate information on the volume concentration of aerosol particles in air.

During sampling of air under field conditions it is necessary to try to match conditions of isokineticity - the main rule ensuring the necessary reliability of analysis results. Isokinetic sampling of air can be ensured by using the May cascade impactor, the automatic Herst trap and other instruments. Application of these investigation methods will make it possible to determine with satisfactory accuracy the quantity of aerosol particles in the investigated volume of air by the subsequent visual method of counting.

The most reliable information on the content of microorganisms in air - i.e., on the concentration of particles of a bacterial aerosol - can be provided by instruments in which the experimental results do not depend on wind speed or particle size. For this purpose aerosol traps constructed on the principle of cutting out a definite volume of air with subsequent sedimentation of all aerosol particles onto a surface covered with nutrient medium are of special suitability.

On should consider to be most promising those methods of studying bacterial seeding of the air which make it possible to

carry out around-the-clock continuous sampling with subsequent or simultaneous sampling with growing of a culture. When such research methods are used it is possible to obtain both indoors and out information on the dynamics of aerial propagation of aerosol particles containing different microorganisms.

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